AD_____

AWARD NUMBER: W81XWH-07-1-0287

TITLE: Cortical-Cortical Interactions and Sensory Information Processing in Autism

PRINCIPAL INVESTIGATOR: Mark Tommerdahl, Ph.D.

CONTRACTING ORGANIZATION: University of North Carolina at Chapel Hill
Chapel Hill, NC 27599

REPORT DATE: April 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 April 2011	Annual	1 Apr 2010 – 31 Mar 2011

4. TITLE AND SUBTITLE

Cortical-Cortical Interactions and Sensory Information Processing in Autism

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-07-1-0287

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Mark Tommerdahl, Ph.D.

E-Mail: tommerda@med.unc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
There are two specific aims or categories of deliverables to be accomplished as tasks in this DoD sponsored research: The first specific aim is the hardware design and fabrication of a portable tactile diagnostic stimulator that can be used for the assessment of the cerebral cortical health of neurologically compromised subjects – in particular, those subjects with autism. The second specific aim is the development of tactile discriminative protocols that will be used for the evaluation of the differences in cerebral cortical function between subjects with and without autism. In this first year of research, all the milestones listed for Y01 in the Statement of Work were met.

15. SUBJECT TERMS
Autism, functional Magnetic Resonance Imaging, Central Nervous System, Two Point Discriminative

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	33	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction

There are two specific aims or categories of deliverables to be accomplished as tasks in this DoD sponsored research: The first specific aim is the hardware design and fabrication of a portable tactile diagnostic stimulator that can be used for the assessment of the cerebral cortical health of neurologically compromised subjects – in particular, those subjects with autism. The second specific aim is the development of tactile discriminative protocols that will be used for the evaluation of the differences in cerebral cortical function between subjects with and without autism. In this fourth year of research, all the milestones listed for Y04 in the Statement of Work were met, with some revisions or extensions of the original proposal. To summarize, we had originally proposed to continue to revise the two-point stimulator that we had developed. However, because our progress exceeded our original expectations in Y03, we advanced the prototype to the stage where we actually built a device which is not only more portable than originally proposed but has much more functionality. Our newest prototype is a 4 point ergonomically designed stimulator (fits any adult sized hand). This improvement in design allows for the new stimulator an additional two versions of the two-point vertical displacement vibrotactile stimulator to address all questions previously addressed with two point stimulation as well as allows us to develop new protocols that utilize 4 fingers. The 4 point stimulator developed in Y03 was further modified in Y04 to be more ergonomic and a new prototype was also designed and fabricated that is magnet compatible. In other words, we have developed a 4 point stimulator that can be used in imaging systems (fMRI, MEG and MRS imaging). Additionally, both the two-point vertical displacement vibrotactile stimulator and the new prototype were used to assess and establish baseline values of the effects of conditioning stimuli on spatial and temporal integration in healthy subjects. Subjects with autism were also recruited and studied using the same protocols and the results from those studies show distinct differences in healthy and autistic subjects. The final milestone - to conduct studies to establish baseline measures of the impact of spatial and temporal integration – was also achieved. The methods that have been and are being developed via tactile sensory diagnostics allow for objective assessment of neurophysiological functional connectivity and could prove to be effective tools for noninvasive assessment of cerebral cortical function.

Milestone #1: *Design and fabrication of a portable diagnostic stimulator*

Contemporary methods for applying multi-site vibratory stimuli to the skin typically involve the use of two separate vibrotactile stimulato rs, which can lead to difficulty with positioning of stimuli and in ensuring that stimuli are delivered perfectly in phase at the same amplitude and frequency. Prior to Y01, we reported a two-point stimulator (TPS) that was developed in order to solve the problem of delivering two-point stimuli to the skin at variable distances between the sites of stimulation. Based on the original TPS, we designed and fabricated a new stimulator in Y01 with four significant improvements over our original device. First, the device is portable, lightweight and can be used in a variety of non-laboratory settings. Second, the device consists of two independently controlled stimulators which allow delivery of stimuli simultaneously to two distinct skin sites with different amplitude, frequency and/or phase. Third, the device automatically detects the skin surface and thus allows for much better automated control of stimulus delivery. Fourth, the device is designed for rapid manufacture and, therefore, can be made readily available to other research (non-laboratory) settings. An additional significant revision of this device was made in Y02 that resulted in the device being more portable (this version is referred to as the CM-2). In this Y02 version, the DAQ interface was moved into the CM-2, and the only components that are necessary for subject testing are the CM-2 and a laptop. In Y03, we re-configured the device to fit into ergonomic

housing and expanded the capability from 2-site to 4-site stimulation (model CM-4). In Y04, the system was made more ergonomic, software and hardware were further refined, and a new model was made (identical to the CM-4) that is magnet compatible and can be used in imaging studies.

Description of the Device

The Cortical Metrics (CM-1; see Figure 1) stimulator was developed in our laboratories for use in the series of experiments described in this report. The system was designed using state-of-the-art rapid manufacturing technology to allow multiple identical systems to be built and used in different locations. Also, the use of rapid manufacturing permitted very rapid design evolution, thereby potentiating the production of special fixtures and changes to geometry as needed for special applications, such as pediatric sizing or the use of special mounting hardware to adapt to existing equipment. The flat plates of the exterior housing and other components of approximately planar geometry are direct manufactured using laser-machined 6mm acrylic sheet, cut on a 120 W att CO_2 laser engraving system, model number X660 (Universal Laser Systems, Scottsdale, AZ). The more complex housing and internal mechanism components are direct manufactured from polycarbonate (PC), by fusion deposition modeling (FDM) on a StrataSys Titan T-1 FDM (StrataSys, Inc., Eden Prairie, MN). All housing and mechanism components and assemblies were solid modeled prior to fabrication using SolidWorks solid modeling software (SolidWorks Corporation, Concord, MA).

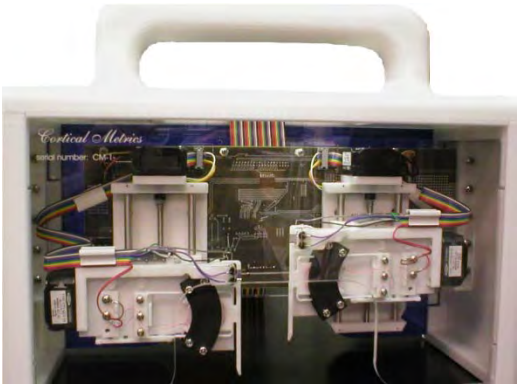


Figure 1: First Version of Portable Tactile Diagnostic Stimulator

The electronics were designed using free CAD software from ExpressPCB (www.expresspcb.com). The printed circuit boards were manufactured using the resulting CAD files, also by ExpressPCB. The electronics employ 5 Microchip microcontrollers; four as dedicated motor controllers for the stepper motors and one as a central controller for the entire system. The hybrid circuit includes signal amplifiers for the position sensors, an analog controller to allow either "force" or "position" control of each VCA motor and tip, a tunable analog PID controller for position control of each tip, and a bipolar push-pull high-current op-amp output stage to drive each VCA motor. This configuration allows each VCA motor to be positioned and driven independently, while coordinated in terms of relative position (x-axis separation between the tips), tip-to-skin mechanical preload, tip vibration amplitude, frequency content, and phase.

The user interface is flexible, allowing several modes of operation. In the simplest mode, used for this series of experiments, a 40-pin ribbon cable connects the internal control logic and analog waveform circuitry directly to a National Instruments data acquisition system (NI DAQ

USB-6251). Tip x and z positions, feedback adjustment, and tip vibration waveforms are generated by a laptop operating NI LabVIEW 7.1 which interfaces to the device using the parallel data cable via the NI DAQ system. In the second configuration, not used in this study, the stimulator system interfaces directly with the laptop via USB, and the intervening NI DAQ system and parallel cable are not needed. The first, simpler configuration was employed in this study because of the ease and convenience of developing tip stimulation waveform protocols using the NI DAQ analog output functions. In Y02, the external DAQ was replaced with an DAQ board internal to the CM-2.

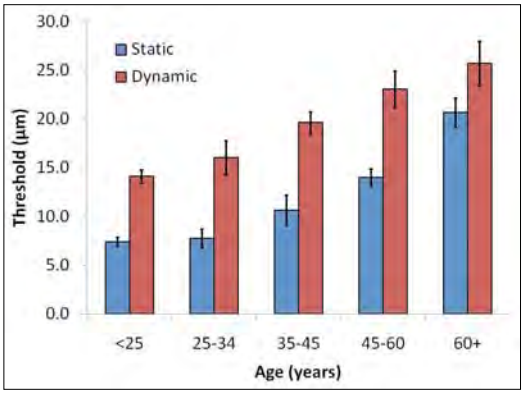
In Y03, we initiated development of a 4 point stimulator device. To summarize, the above-described description of the device design of the two-point stimulator (CM-1) was reconfigured in housing that would allow for four-point stimulation. While the CM-1 device was still being utilized, a complete remodel of the device was initiated in order to make the system more portable and more ergonomic. The prototype of this device is pictured at the left. Note that in addition to being smaller, there are 4 sites of finger tip stimulation and the position of the stimulators is adjustable. Debugging of this device has been completed and we have begun using the device for data collection. In addition to improvements in ergonomics and portability, the device is much easier and more affordable to reproduce.

In Y04, we further improved the ergonomics of the device and began to re-design the configuration so that the entire system could fit into the forearm support. Note figure at left: ergonomics are improved by the change in shape of the forearm support. It is the forearm support pictured in the inset that will eventually house the entire system after we have completed re-design of the internal components of the main unit.

Milestone #2: *Development of tactile discriminative protocols*

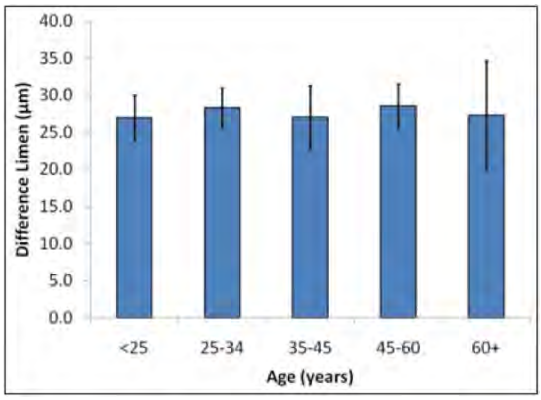
The CM-4 tactile stimulating device allows for simultaneous delivery of skin stimuli from four independently controlled stimulators that are mounted in a small, portable package that can be used on virtually any desktop. In our Y01 and Y02 report, we demonstrated that simultaneous amplitude discrimination tracking is a task that can be completed reliably and efficiently with the CM-1 and CM-2 systems, respectively. With the CM-4, we have achieved significant proof-of-concept that the all the protocols that the CM-1 and CM-2 were capable of can be delivered with the CM-4. Additionally, in Y04, we were able to demonstrate that the CM-4 is a reliable tool for assessing amplitude discriminative capacity of human subjects. One of our objectives in this work is determining what metrics are obtained can be reliable indicators of CNS health, regardless of age. Traditionally, sensory thresholds have been used as metrics for evaluating a number of conditions by clinicians, but they are very sensitive to age, and thus, cannot be reliable indicators for CNS health.

Our approach to threshold detection is slightly altered from the traditional approach. The majority of published studies report thresholds using one stimulus site, and subjects generally indicate whether they feel "something" or "nothing". Utilization of two stimulus sites allows for the subject to be questioned as to where they thought the stimulus was, and ultimately generates a more accurate answer.



Our working hypothesis is that a centrally mediated measure will remain effectively constant across a subject population, provided that the CNS is healthy, although we would expect there to be a wide range in peripherally mediated measures. To demonstrate this concept, we examined thresholds across the healthy adult population. Due primarily to changes in skin physiology, detection thresholds go up (or sensitivity goes down) with age. Note in the graph at the right how threshold goes up with age. Also note that two types of threshold measures are shown – a "static" threshold in which a two-forced choice paradigm is used to track threshold and a "dynamic" threshold in which a subject has to detect a modulated stimulus (increasing in size from zero to threshold level; also a two forced choice paradigm). The difference in the two results is currently postulated to be the result of feed-forward inhibition at the level of layer IV in SI cortex, and thus, this metric that we have developed could be a very sensitive measure for detecting changes in the CNS.

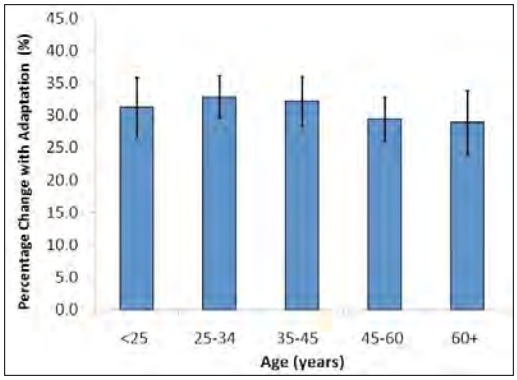
In order to make the diagnostic tests more robust, we sought to improve the signal to noise ratio by increasing the stimulus size and having subjects make comparisons between two stimuli.



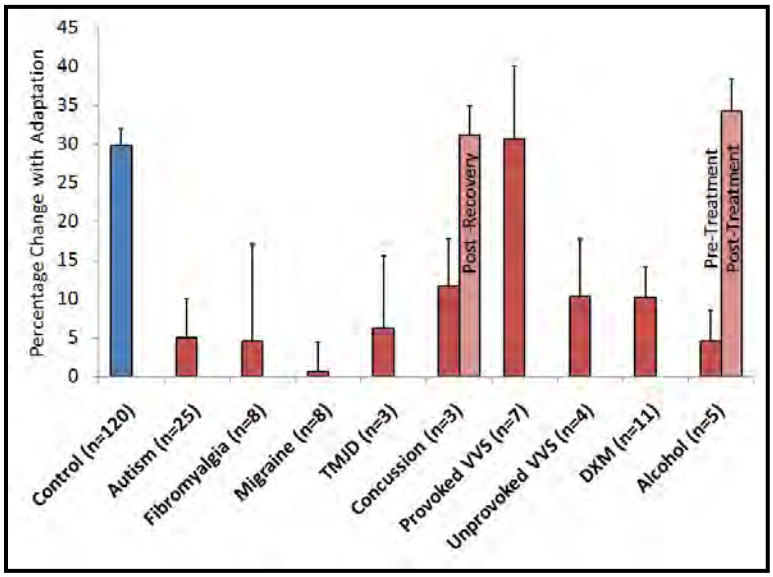
To test the idea that comparison of two adjacent stimuli is relatively stable in healthy populations, an amplitude discrimination task in which both stimuli were delivered simultaneously to adjacent finger tips (D2 and D3) was performed on healthy subjects across a wide age spectrum. This measure was effectively constant across all age groups (see Figure at left). It should be noted that, in order to maximize signal to noise ratio, we conduct this amplitude discrimination task at supra-threshold levels. This allows us to deliver the same size stimuli to all subjects and thus, although threshold variation (discounting for subjects with very significant peripheral neuropathy) is in the range of 10-30 microns, all subjects are able to effectively compare stimuli that are 100 microns or greater. The problem with amplitude discrimination as a potential clinical measure is that it is often *too* robust: Comparison of this measure between healthy controls and a number of subject populations demonstrated little significant difference. For this reason, we chose to examine more closely how different factors, and their underlying mechanisms, would impact amplitude discrimination capacity. Thus, we took advantage of the robustness of amplitude discriminative capacity at these stimulus parameters in multiple subject populations by utilizing this measure as a baseline for each subject, and the critical measure for clinical purposes will be how this measure is altered under different experimental conditions.

Randomly applying a conditioning stimulus to one of the two skin sites before the amplitude discrimination task significantly alters a subject's ability to determine the actual difference between the two stimuli, and the impact that the conditioning stimulus has is duration dependent

(between 0.2 and 2 secs; (for discussion, see Tannan et al., 2007a, 2008; Tom merdahl et al, 2007a, 2007b, 2008; Francisco et al, 2008; Zhang, et al, 2009).). This finding suggested that the method could be viewed as a reliable indicator of the influence of adapting stimuli on central nervous system response, as changes in the peripheral response are not significantly changed at these short stimulus durations. Simply stated, the reason that subjects get worse with conditioning stimulation to one of the stim ulus sites is because the subsequent stim ulus, which is used for comparison, now feels smaller than it really is. This creates an illusory effect which appears to be relatively constant across healthy populations regardless of age (see Figure at right).



When this m easure is exam ined across a num ber of subject populations (w e initiated a number of pilot studies in Y04 by providing prototypes of the device to clinical researchers in a number of areas), we do see significant deviations from control values. To sum marize, the chart below demonstrates that this centrally m ediated measure deviates from the control values for subjects with autism (Tannan et al, 2008), chroni c pain (fibrom yalgia, migraine, TMJD) and concussion. Ingestion of 60m g of dextrom ethorphan (DXM) also leads to a reduction in the impact of the adapting stim ulus (Folger et al, 2008) as does chronic use of alcohol. Values that appear in the control range are post-recovery of concussion (3-7 days after concussion) and post-treatment of alcoholism (12 w eeks of sobriety; initial m easure was after 1 day of sobriety and BAC was zero). "Provoked VVS" is considered peri pherally mediated (and exhibits near control values) while "unprovoked V VS" is a chronic pain condition w hich is thought to be centrally mediated (Zhang et al, 2011). The significance of the VVS study is that distinct differences in information processing capacity (or central sens itization) of the two groups was detected by utilizing sensory testing m ethods on a body region (the finger tips) that w as not impacted by the subjects' affliction (pelvic pain). In other w ords, pelvic pain had little or no im pact on subjects' thresholds, but it did have an impact on the subjects' centrally mediated neuroadaptation.



At first glance, the above-described method appears fairly non-specific: virtually all the data obtained from subjects with some type of centrally mediated neurological disorder is impacted. Adaptation, which encompasses the ability to quickly adjust to one's environment, involves a number of m echanisms. The protocols that have been designed to obtain neuroadaptation metrics were optimized to enable objective evaluation of the neuronal communication between adjacent and near-adjacent regions within sensory cortex that is widely recognized to be essential to normal sensory function.

In other words, this "functional connectivity" between two adjacent or near-adjacent cortical regions is indicative of overall CNS functional connectivity. The mechanisms required for this communication include neurotransmission mediated by the inhibitory neurotransmitter GABA and by NMDA receptors, and interactions / interdependencies between neurons and glial cells. These particular processes have been demonstrated to have a significant impact on centrally mediated cortical adaptation, and thus, this type of diagnostic test could prove useful as a quick (2-3 minutes), reliable and efficient means for assessing CNS health. Diagnostic tests aimed at extracting more specific information about functional connectivity have been and will continue to be developed: This grant mechanism has provided an important catalyst to a new methodology which could prove to be useful in a number of clinical fields. Metrics obtained from multiple subject populations not only gives us information about the information processing capacities of those particular groups, but it gives us further insight into how the new metrics relate to the cortical information processing capacity of the autism population.

Key Research Accomplishments

- Evolution of a portable two-point stimulator to a four-point stimulator
- Development of novel protocols of cortical information processing assessment
- Collection of baseline data from healthy subjects for spatial and temporal integration tasks
- Collection of baseline data from autism subjects for spatial and temporal integration tasks
- Demonstration that a number of baseline metrics are reliable across the age spectrum

Reportable Outcomes

Peer Reviewed Manuscripts, accepted for publication:

Zhang Z, Zolnoun D, Francisco E, Holden JK, Dennis RG, Tommerdahl M (2011) Altered central sensitization in subgroups of women with vulvodynia. The Clinical Journal of Pain. *In Press.*

Holden JK, Francisco EM, Zhang Z, Baric C and Tommerdahl M (2011). An Undergraduate Laboratory Exercise to study Weber's Law. Journal of Undergraduate Neuroscience Research, 9:2.

Tommerdahl M, Dennis RG and Favorov OV (2010). Non-invasive CNS Diagnostics. Proceedings of the 27th Army Science Conference.

Manuscripts in Review:

Tommerdahl M and Favorov OV (2011) The role of cortical modularity in tactile information processing: measuring CNS processing deficits in autism.

Francisco E, Holden J, Zhang Z, Favorov O and Tommerdahl M (2011). Rate dependency of vibrotactile stimulus modulation. *In Revision.*

Zhang Z, Francisco E, Holden JK, Dennis RG, Tommerdahl M (2011) Cortical-cortical interactions in the aging population. *Submitted.*

Presentations:

Tommerdahl, M. Non-Invasive CNS Diagnostics. 27th Army Science Conference, Orlando, Florida. December 1, 2010.

Conclusion

The development of unique quantitative sensory testing methods was made possible by the design and fabrication of a portable two-point vibrotactile stimulator (and recently, subsequent design and fabrication of a portable four-point vibrotactile stimulator). The sensory testing methods and apparatus that were developed were designed to enable objective evaluation of the elaborate neuroanatomical connectivity that sub serves the neuronal communication between adjacent and near-adjacent regions within sensory cortex that is widely recognized to be essential to normal sensory function. There have been several significant findings in the early stages of applications of these methods in our autism research. First, results comparing the spatial localization ability of subjects with autism vs. controls demonstrated that although cutaneous localization performance of adults with autism is significantly better than the performance of control subjects, tactile spatial discriminative capacity remained unaltered in the same autism subjects when examined after the duration of adapting stimulation was increased although significant improvement was observed in controls. Second, results comparing the ability of subjects with autism to discriminate between the intensity of two simultaneously delivered stimuli demonstrated that although autism subjects were equal to or better than control subjects at short duration discrimination tasks, conditioning stimuli delivered prior to a task had no impact on the autism subjects' ability to discriminate although this conditioning had significant impact on control subjects' perception. Both the failure of prior history of tactile stimulation to alter sensory percepts in adults with autism, and the better-than-normal perceptual performance of adults with autism in these tasks, were concluded, in the above-mentioned studies, to be attributable to both the smaller than normal minicolumn width observed in autism subjects and the deficient cerebral cortical GABAergic inhibitory neurotransmission characteristic of this disorder. A third study examined the effective short-range functional connectivity of subjects with autism vs. neurotypical controls, and significant differences were found between controls and autism subjects in the influence that synchronizing stimuli have on sensory perception. An important emerging concept in autism research is the role of dysfunctional neural synchrony, and it was speculated from these recent findings that the local functional connectivity that normally sub serves long range connectivity and synchrony could be a result of the abnormal minicolumn architecture that has been previously reported by others. One unifying theme of these findings is the role that cortical modularity plays in sensory information processing, and in autism, cortical modularity is disrupted to an extent that significant quantifiable deficits in sensory information processing can be detected. In terms of practical application, future work could mean that the methods that we are developing could be used for both basic diagnostic applications as well as determination of efficacy of intervention. It should be noted that the utility of these methods is not limited to their application to the field of autism. The methodology that was developed under this grant mechanism has enormous potential to provide a number of clinical fields with low cost, high throughput, biologically based, efficient and effective diagnostic tools.

References

Tannan, V., Holden, J.K., Zha ng, Z., Baranek, G., & Tommerdahl, M.A. (2008). Perceptual metrics of individuals with autism provide evidence for disinhibition., A utism Research. Aug; 1(4): 223-30.

Tannan, V., Simons, S., Dennis, R.G., & Tommerdahl, M. (2007a). Effects of adaptation on the capacity to differentiate sim ultaneously delivered dual-site vibrotactile stim uli. Brain Res., Jun 18; 1154:116-23.

Tannan V, Dennis RG, Zhang Z, and Tom merdahl M. (2007b) A portable tactile sensory diagnostic device. J Neurosci Methods, Aug 15; 164(1): 131-8.

Tannan, V., Whitsel, B.L., & Tommerdahl, M.A. (2006). Vibrotactile adaptation enhances spatial localization. Brain Res, 1102, 109-16.

Tannan V, Dennis RG, Tom merdahl M (2005a) A novel device for delivering two-site vibrotactile stimuli to the skin. J Neurosci Methods June.

Tannan V, Dennis R, Tom merdahl M (2005b) Stim ulus-dependent changes in spatial acuity. Behavioral and Brain Functions Oct 10.

Tommerdahl, M., Tannan, V., Cascio, C.J., Baranek, G.T., & Whitsel, B.L. (2007a). Vibrotactile adaptation fails to enhance spatial localization in adults with autism. Brain Res, 1154, 116-23.

Tommerdahl, M., Tannan, V., Z achek, M., Holden, J.K., & Favorov, O.V. (2007b). Effects of stimulus-driven synchronization on sensory perception. Behav Brain Funct, 3, 61.

Tommerdahl M, Tannan V, Holden JK, Barane k GT. (2008) Absence of stim ulus-driven synchronization effects on sensory percep tion in autism : Evidence for local underconnectivity? Behavioral and Brain Functions, 4:19.

Zhang Z, Francisco E, Holden JK, Dennis RG, Tommerdahl M (2009) The impact of non-noxious heat on tactile information processing. Brain Research, Dec 11; 1302:97-105.

Appendices

The following papers are included in the Appendix:

Zhang Z, Zolnoun D, Francisco E, Holden JK, Dennis RG, Tommerdahl M (2011) Altered central sensitization in subgroups of women with vulvodynia. The Clinical Journal of Pain. *In Press.*

Holden JK, Francisco EM, Zhang Z, Baric C and Tommerdahl M (2011). An Undergraduate Laboratory Exercise to study Weber's Law. Journal of Undergraduate Neuroscience Research, 9:2.

Tommerdahl M, Dennis RG and Favorov OV (2010). Non-invasive CNS Diagnostics. Proceedings of the 27th Army Science Conference.

NON-INVASIVE CNS DIAGNOSTICS

Mark Tommerdahl*, Robert G. Dennis and Oleg V. Favorov
University of North Carolina
Chapel Hill, NC 27599

ABSTRACT

A large number of neurological disorders (neurodegenerative, neurodevelopmental or trauma induced) are difficult to diagnose or assess, thus limiting treatment efficacy. Existing solutions and products for this need are costly, extremely slow, often invasive, and in many cases fail to definitively (and quantitatively) diagnose or assess treatment. Our innovative low-cost sensory testing device non-invasively assesses the central nervous system (CNS) health status in minutes for numerous patient populations that are currently difficult to diagnose or assess. Based on pilot data (currently an ontological database of over 500 subjects), the system can be used to enable clinicians to have a much better view of a patient's CNS health status. The diagnostic system delivers a battery of sensory based (tactile) tests that are conducted rapidly – much like an eye exam with verbal feedback – and are designed around the concept of measuring how much the CNS is impacted by a particular neurological disorder. Design and validation of the perceptual metrics was/is accomplished via correlative studies that compare non-invasive observations of human sensory percepts with non-human primate neurophysiological studies.

INTRODUCTION

There are countless reasons that a person's blood pressure could be high: hardening of the arteries, too much salt in the diet, kidney malfunction, obesity, etc., could all be one of many of the potential culprits that cause high blood pressure. The long list of things that could lead to high blood pressure would seemingly deter us from using it as a standard measure of health, since any of a number of factors could be what led to the deviation from normative values and thus, the measure seems somewhat nonspecific. However, it is generally regarded as a starting point for a physician to determine what, if any, action should be taken to return a patient to cardiovascular health.

Could such a non-invasive procedure exist for evaluating a patient's central nervous system (CNS)? Sensory perception relies on many facets of the CNS for a patient or subject to perform well on (or within a "normal" range). First, it requires that the peripheral nervous system (PNS) is, for the most part, intact. Second, transmission of the signal must reach the sensory cortex (in the case of the somatosensory system, via the spinal cord) with reasonably good fidelity. Third, processing within the cerebral cortex must be capable of spatially and temporally integrating information that it has received, and this typically requires multiple levels of processing – both in the primary sensory cortex as well as at cognitively higher levels.

The somatosensory system is ideally suited for the design of a CNS diagnostic system. First, the organization of the system is such that adjacent skin regions project to adjacent cortical regions (i.e., it is somatotopic). Second, ambient environmental noise in the system can be easily controlled (i.e., it is less likely that a patient will be exposed to distracting tactile input than auditory or visual input). Third, the somatosensory system is the only sensory system that is highly integrated with the pain system, and this is often an important aspect of a patient's diagnosis.

As a first step towards developing quantitative sensory testing methods that could non-invasively detect systemic alterations in the CNS, we designed and fabricated a portable multi-site stimulator. The stimulator pictured below (Figure 1; CM4 is fourth Cortical Metrics

Figure 1. CM4 vibrotactile stimulator.

model) can be used to deliver vibrotactile stimuli to four digit tips (see inset to Figure 1). Typically, the mechanical stimuli that are delivered to the skin are sinusoidal in nature, and the stimulator is capable of delivering amplitudes in the range of 0 – 1000 microns and frequencies in the range of 0 – 200Hz. Stimuli can be

delivered independently to each finger tip, and the temporal sequences of stimuli, as well as the amplitude, frequency, and duration, are computer controlled via USB interface. Prior models of the device and its design have been previously reported (Tannan et al, 2005a, 2007b).

In parallel with the development of the stimulator, we designed a number of unique sensory testing protocols aimed at extracting information exclusively about the CNS in an efficient and reliable manner. In this paper, we describe the evolution of one of those measures. First, a description of how traditional clinical measures (e.g., tactile sensory detection thresholds) can be obtained is provided. These types of tests provide sensory information that is analogous to that normally obtained with a vision (e.g., eye chart exam) or hearing test. Second, we briefly describe the neurophysiological basis for comparing two simultaneously delivered stimuli, and sample data from this type of sensory test is also provided. Third, a description of how a measure can be impacted by an individual's ability to adapt to tactile stimuli (on the order of a second or less) and can be quantified is presented. In this section, we provide evidence that this type of metric – which we term as a "cortical metric" – has potential for providing clinicians with diagnostic information as well as a means for assessment of treatment efficacy for a number of neurological disorders.

SENSORY THRESHOLDS

Tactile detection thresholds are typically collected by clinicians because sensitivity to a tactile stimulus generally increases with a neurological alteration. However, threshold measures are most useful if baselines are recorded – i.e., if a subject's threshold is acquired before something impacts his/her neurological health, then comparisons can be directly made. Without baseline measures, there can be a great deal of variability from one subject to another, primarily due to skin physiology (e.g., the callused hands of a manual laborer could result in higher than normal detection thresholds).

Our approach to threshold detection is slightly altered from the traditional approach. The majority of published studies report thresholds using one stimulus site, and subjects generally indicate whether they feel "something" or "nothing". Utilization of two stimulus sites allows for the subject to be questioned as to where they thought the stimulus was, and ultimately generates a more accurate answer.

Our working hypothesis is that a centrally mediated measure will remain effectively constant across a subject population, provided that the CNS is healthy, although we would expect there to be a wide range in peripherally mediated measures. To demonstrate this concept, we examined thresholds across the healthy adult population. Due primarily to changes in skin physiology, detection thresholds go up (or sensitivity goes down) with age. Note in the graph in Figure 2 how threshold goes up with age. Also note that two types of threshold measures are shown – a "static" threshold in which a two-forced choiced paradigm is used to track threshold and a "dynamic" threshold in which a subject has to detect a modulated stimulus (increasing in size from zero to

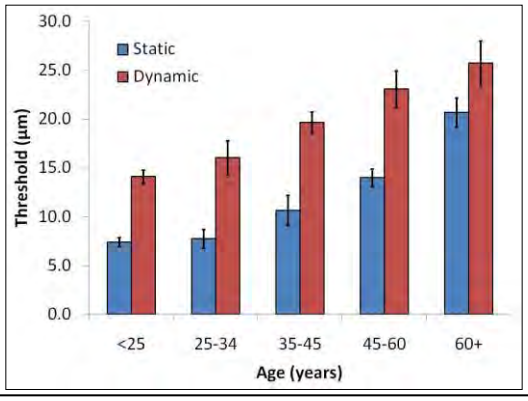


Figure 2. Tactile detection thresholds across an age spectrum. Note increase in threshold with increasing age.

threshold level; also a two forced choice paradigm; see Zhang et al 2009 for description). The difference in the two results is currently postulated to be the result of feedforward inhibition at the level of layer IV in SI cortex, and this hypothesis, modeled in Figure 3, is currently being investigated experimentally.

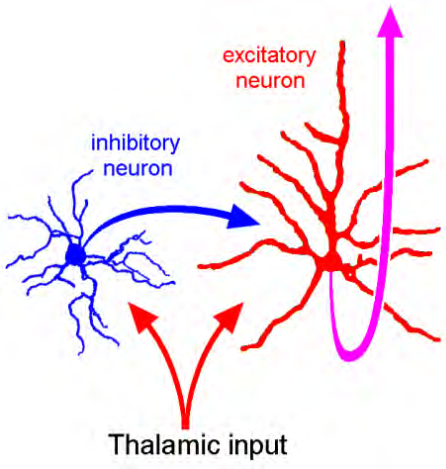


Figure 3. Feedforward model of inhibition.

In our current feedforward model of dynamic threshold processing, we postulate that subthreshold inputs transmitted from the skin to cortex via thalamus result in activity driven inhibition. This inhibition subsequently raises the threshold, and the next stimulus – which is slightly larger than the previous stimulus – remains to be sub-threshold and in turn, causes another subsequent increase in inhibition. Eventually, the magnitude of the stimulus is increased enough to overcome the increased threshold. Deficiencies in the underlying mechanisms that support this feedforward inhibition would result in significant increases or decreases in the differences observed between static and dynamic thresholds.

BASIS FOR CNS TESTING: GENERATING *CORTICAL METRICS.*

A biopsy is often used to examine the viability of a biological tissue that is suspected to be diseased or injured. While this method is invasive, it is nevertheless quite effective as a means of putting one piece of a suspected tissue under a high degree of scrutiny. The underlying premise in that form of testing is that all pieces of the organ tissue have been afflicted by trauma, disease and/or injury in a similar manner. For this reason, we hypothesized that we could develop novel means to "non-invasively biopsy" the cerebral cortex of subjects/patients that have undergone some systemic neurological alteration. The techniques are based on strong correlations that have been observed (utilizing both human and animal studies in parallel) between cerebral cortical activity and perceptual measures that are evoked by tactile stimulation. Thus, the combinations of stimuli that are delivered to the skin generate sensory percepts that can be predominately accounted for within a relatively small region of cortex. Although this region of parietal cortex could be considered limited in scope, much of the basic cortical circuitry that is utilized throughout the entire cerebral cortex can be found in this "slice" and studying the interactions that take place in this slice can give explicit metrics about the functional connectivity that exists between adjacent and near-adjacent cortical regions. For example, comparison of the two images in Figure 4 that were evoked by the same conditions of electrical stimulation in the cortical slice demonstrate the difference that a small amount of GABA (gamma aminobutyric acid) antagonist (2 μM bicuculine) will have on the spatial distinction between the two near-adjacent cortical columns that have been activated (dark regions indicate areas of high neural activity; for more thorough experimental description, see Kohn et al, 2000). Similar experiments (both *in vivo* and *in vitro*) have shown that (1) GABA agonists improve the contrast between these cortical areas, (2) this contrast can also be improved with short-duration (1-5 sec) repetitive stimulation (a function of cerebral cortical neuroplasticity), (3) the improvements made by repetitive stimulation can be blocked with NMDA (N-methyl-d-aspartate) receptor antagonists, and (4) reversibly blocking glial activity will significantly impact the cortical response (for limited referral, see Whitsel et al, 1999).

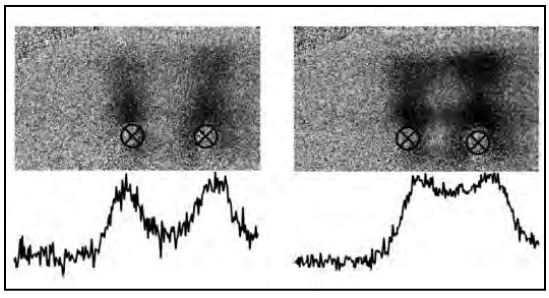


Figure 4. Top: images of activity evoked in sensorimotor cortical slice of the rat. Circled "X" denotes location of electrical stimulus. Bottom: Spatial histograms of above images.

The significance of Figure 4 is that delivery of two identical stimuli to the same cortical sites yield two distinctly different results under different experimental conditions. In the absence of a GABA antagonist, the two cortical regions are easily contrasted, as depicted by the cross-sectional histogram. With lower levels of GABA (in this case, due to the GABA antagonist), the contrast between the two stimulated sites is greatly reduced. Thus, this would predict that a subject, with diminished GABA levels (as is the case for a number of neurological disorders and/or traumas), would have more difficulty distinguishing the difference between two stimuli presented to skin sites that project to adjacent or near adjacent areas in the cortex than a subject without diminished or altered GABA levels.

To test the idea that comparison of two adjacent stimuli is relatively stable in healthy populations, an amplitude discrimination task in which both stimuli were delivered simultaneously to adjacent finger tips (D2 and D3) was performed on healthy subjects across a wide age spectrum. This measure was effectively constant across all age groups (see Figure 5). It should be noted that, in order to maximize signal to noise ratio, we conduct this amplitude discrimination task at supra-threshold levels. This allows us to deliver the same size stimuli to all subjects and thus, although threshold variation (discounting for subjects with very significant peripheral neuropathy) is in the range of 10-30 microns, all subjects are able to effectively compare stimuli that are 100 microns or greater.

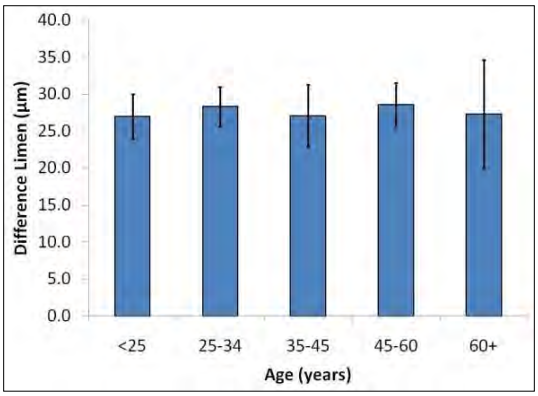


Figure 5. Average difference limens (DLs) recorded across the age spectrum. Note the absence of change with age as was the case in Figure 3.

In order to validate the concept that amplitude discriminative capacity is directly related to SI cortical activity, we compared the results from our human perceptual studies to non-human primate imaging studies. This was accomplished by investigating the SI response to different amplitudes of vibrotactile stimulation utilizing the technique of optical intrinsic signal (OIS) imaging in nonhuman primates. We found that an increase in the amplitude of the stimulus corresponded with the increase in absorbance evoked within the region of SI cortex that receives its input from the stimulated skin field (Simons et al. 2005; Simons et al. 2007). The relationship between the maximal change in absorbance and stimulus amplitude was characterized by a near-linear function within the range of amplitudes studied (50-400 µm). Measurement of the spatial extent of the activated SI region, on the other hand, showed that higher amplitudes of stimulation did not produce a more extensive region of SI activation. Instead, as the amplitude was increased, average peak absorbance within an ~2 mm diameter SI region increased with the amplitude of stimulation, and the region of surrounding cortex underwent a prominent decrease (frequently to levels well below background) in absorbance. In order to directly compare the two principal findings of that study – the relationship of absorbance evoked by different amplitudes of stimulation and the apparent lack of correspondence of the spatial extent with amplitude of stimulation – we directly compared the DLs obtained from human sensory testing with those two entities. The results from those comparisons are shown in Figure 6 and demonstrate that there is a very strong correlation ($R^2 = 0.9971$) between the DLs obtained at each standard amplitude and the neural activity evoked at each amplitude. On the other hand, a much weaker (not significant) correlation was observed between the spatial extent of the cortical response and the DLs obtained at the same amplitudes ($R^2 = 0.4542$).

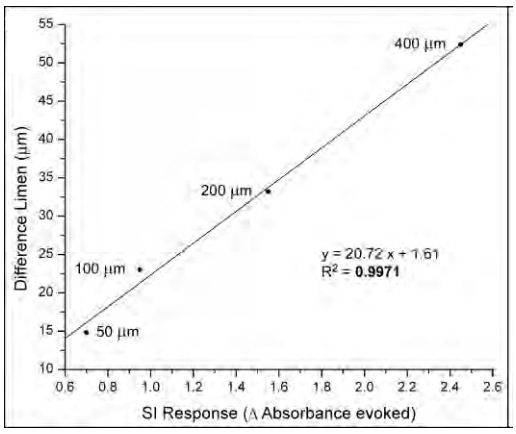


Figure 6. Correlation between human perceptual performance (DLs) and response recorded in SI evoked by same stimulus conditions in non-human primates. Modified from Francisco, et al, 2008.

The problem with amplitude discrimination as a potential clinical measure is that it is often *too* robust: Comparison of this measure between healthy controls and a number of subject populations demonstrated little significant difference (Tannan et al 2008; Zhang et al, 2009; Folger et al, 2008). For this reason, we chose to examine more closely how different factors, and their underlying mechanisms, would impact amplitude discrimination capacity. Thus, we can take advantage of the robustness of amplitude discriminative capacity at these stimulus parameters in multiple subject populations by utilizing this measure as a baseline for each subject, and the critical measure for clinical purposes will be how this measure is altered under different experimental conditions.

REPETITIVE STIMULATION & NEUROADAPTATION METRICS

Repetitive stimulation results in temporally defined changes of cortical activity, the most prominent of which is a reduction in cortical response with extended stimulus duration. At the single cell level, both visual and somatosensory cortical pyramidal neurons undergo prominent use-dependent modifications of their receptive fields and response properties with repetitive stimulation. These modifications can attain full development within a few tens of milliseconds of stimulus onset, and can disappear within seconds after the stimulus ends (visual cortical neurons: (Bredfeldt and Ringach, 2002; Celebrini et al., 1993; Das and Gilbert, 1995; DeAngelis et al., 1995; Dinse and Kruger, 1990; Pack and Born, 2001; Pettet and Gilbert, 1992; Ringach et al., 1997; Shevelev et al., 1998; Shevelev et al., 1992; Sugase et al., 1999); alternatively, for review of short term cortical neuron dynamics in visual cortex: (Kohn, 2007); for review of short-term primary somatosensory cortical neuron

dynamics: (Kohn and Whitsel, 2002; Whitsel et al, 2002)). These rapid changes in cortical neurons are in striking contrast with the relative stability of primary afferent neurons (Whitsel et al, 2000).

Optical imaging studies have also characterized the short-term dynamics of the population-level response of squirrel monkey contralateral primary somatosensory (SI) cortex using different amplitudes and durations of vibrotactile stimulation (Chiu et al., 2005; Simons et al., 2007; Simons et al., 2005). The results of these optical intrinsic signal (OIS) imaging studies demonstrated a strong correlation between the amplitude of 25 Hz vibrotactile (flutter) skin stimulation and the response magnitude evoked in SI. In addition to the systematic changes in the spatial pattern of response in SI that correlated with increases in the amplitude and the duration of the stimulus, increasing the stimulus duration led to differences not only in the peak magnitude of the evoked cortical response, but also in the relative rates of rise and decay of the magnitude of the evoked intrinsic signal. These differences in the rates of rise and decay could impact the refractory period following a stimulus during which the magnitude of the response to a subsequent stimulus is diminished (Cannestra et al., 1998).

The perceptual improvements in spatial discrimination that are normally observed with extended stimulus durations (e.g., Goble and Hollins, 1993; Tannan et al, 2005b, 2006) could be attributed to stimulus-evoked inhibition that surrounds areas of excitation. Single unit studies and imaging studies using voltage-sensitive dyes likewise have shown that excitation in the responding neuronal population is accompanied by the development of a surrounding field of inhibition (Brumberg et al., 1996; Derdikman et al., 2003; Foeller et al., 2005; Wirth and Luscher, 2004). Similarly, imaging studies that have used the OIS have shown that prolonged stimulation of a discrete skin site not only is associated with increased absorbance within the SI region representing the stimulated skin site, but also with decreases in absorbance in surrounding regions (Moore et al., 1999; Simons et al., 2005; Tommerdahl and Whitsel, 1996; Tommerdahl et al., 1999). Regions of decreased absorbance (increased reflectance) such as that described in Figure 7 are widely believed to be indicative of decreases in neuronal spike discharge activity (Grinvald, 1985; Grinvald et al., 1991), possibly resulting from stimulus-evoked inhibition at these locations. There is a great deal of evidence that the suppressed or below-background activity observed to suggest that stimulus-evoked inhibition is responsible for the improvements in discriminative performance that are normally observed with repetitive stimulation. Thus, we would anticipate that a task such as the aforementioned amplitude discrimination, would be sensitive to the duration of stimulation. Utilization of this scientific work has led us to more effectively develop sensory testing methods which detect deficiencies in specific clinical populations.

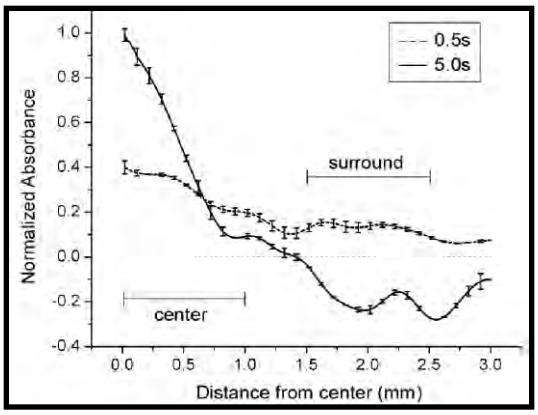


Figure 7. Radial histograms of SI cortical activity, measured via optical imaging. Data at center was at the maximally responding SI cortical territory to the 5 sec stimulus condition (modified from Simons et al, 2007).

Randomly applying a conditioning stimulus to one of the two skin sites before the amplitude discrimination task significantly alters a subject's ability to determine the actual difference between the two stimuli, and the impact that the conditioning stimulus has is duration dependent (between 0.2 and 2 secs; Tannan et al, 2007). This finding suggested that the method could be viewed as a reliable indicator of the influence of adapting stimuli on central nervous system response, as changes in the peripheral response are not significantly changed at these short stimulus durations (for discussion, see Tannan et al., 2007a, 2008; Tommerdahl et al, 2007a, 2007b, 2008; Francisco et al, 2008; Zhang, et al, 2009). Simply stated, the reason that subjects get worse with conditioning stimulation to one of the stimulus sites is because the subsequent stimulus, which is used for comparison, now feels smaller than it really is. This creates an illusory effect which appears to be relatively constant across healthy populations regardless of age (see Figure 8).

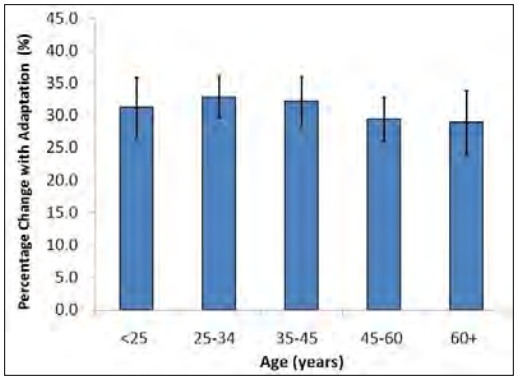


Figure 8. Change in amplitude discrimination DL after conditioning at a single stimulus site.

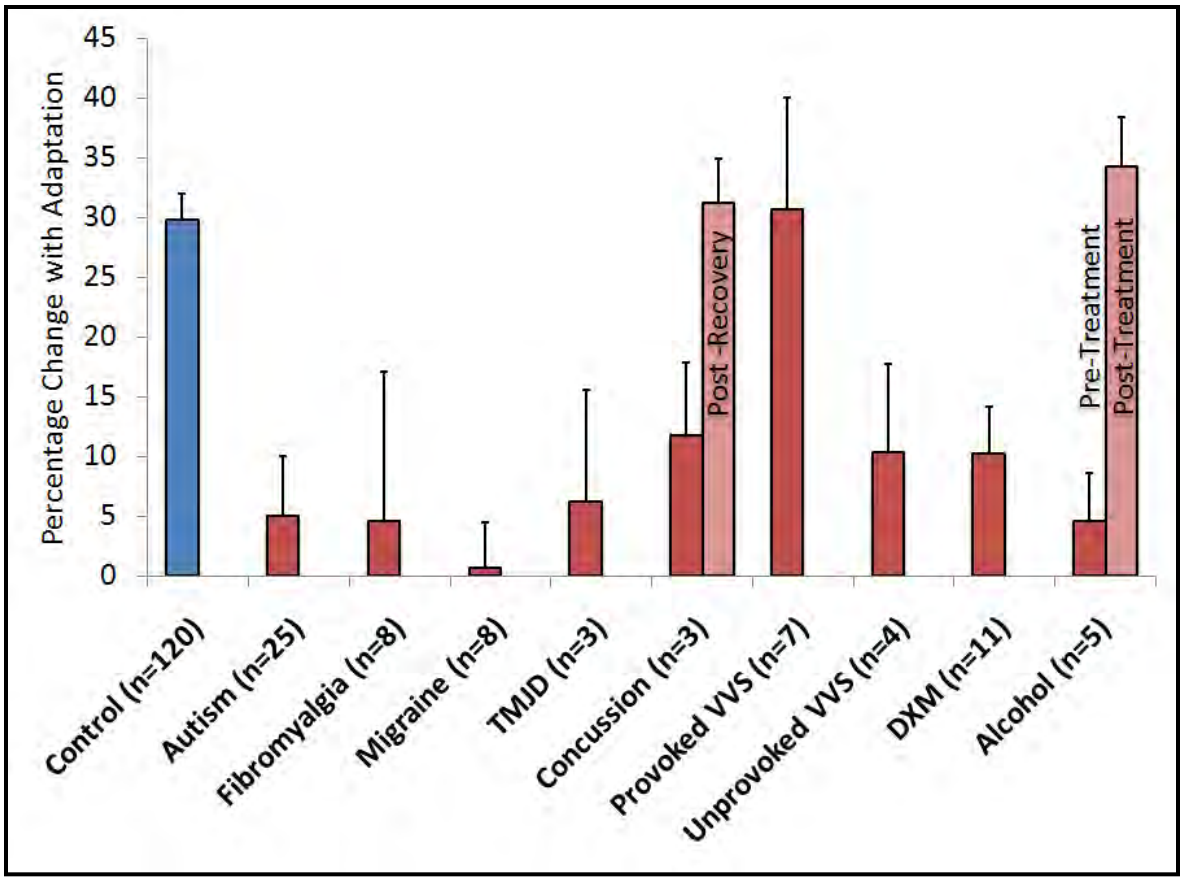


Figure 9. *Neuroadaptation Metrics*: Summary of adaptation metrics obtained from several different subject populations. Note that the amount that several subject populations adapt is much less than that of controls. The two exceptions are provoked VVS (peripherally mediated) and post-treatment of alcohol subjects (measures obtained after 12 weeks of sobriety). Thus, the impact of changes in centrally mediated mechanisms can be detected using a relatively fast vibrotactile methodology. Note that DXM refers to a post-ingestion of 60mg of dextromethorphan in control subjects. Data from control, autism and DXM subjects has been previously reported (Tannan et al, 2007b, 2008; Folger et al, 2008).

When this measure is examined across a number of subject populations, we do see significant deviations from control values. To summarize, the chart in Figure 9 demonstrates that this centrally mediated measure deviates from the control values for subjects with autism (Tannan et al, 2008), chronic pain (fibromyalgia, migraine, TMJD) and concussion. Ingestion of 60mg of dextromethorphan (DXM) also leads to a reduction in the impact of the adapting stimulus (Folger et al, 2008) as does chronic use of alcohol. Values that appear in the control range are post-recovery of concussion (3-7 days after concussion) and post-treatment of alcoholism (12 weeks of sobriety; initial measure was after 1 day of sobriety and BAC was zero). "Provoked VVS" is considered peripherally mediated (and exhibits near control values) while "unprovoked VVS" is a chronic pain condition which is thought to be centrally mediated (Zhang et al, 2010; manuscript in preparation). The significance of the VVS study is that distinct differences in information processing capacity (or central sensitization) of the two groups was detected by utilizing sensory testing methods on a body region (the finger tips) that was not impacted by the subjects' affliction (pelvic pain). In other words, pelvic pain had little or no impact on subjects' thresholds, but it did have an impact on the subjects' centrally mediated neuroadaptation metrics.

At first glance, the above-described method appears fairly non-specific: virtually all the data obtained from subjects with some type of centrally mediated neurological disorder is impacted. Adaptation, which encompasses the ability to quickly adjust to one's environment, involves a number of mechanisms. The protocols that have been designed to obtain neuroadaptation metrics were optimized to enable objective evaluation of the neuronal communication between adjacent and near-adjacent regions within sensory cortex that is widely recognized to be essential to normal sensory function. In other words, this "functional connectivity" between two adjacent or near-adjacent cortical regions is indicative of overall CNS functional connectivity. The mechanisms required for this

communication include neurotransmission mediated by the inhibitory neurotransmitter GABA and by NMDA receptors, and interactions / interdependencies between neurons and glial cells. These particular processes have been demonstrated to have a significant impact on centrally mediated cortical adaptation, and thus, this type of diagnostic test could prove useful as a quick (2-3 minutes), reliable and efficient means for assessing CNS health. Diagnostic tests aimed at extracting more specific information about functional connectivity have been and are continuing to be developed (Tommerdahl et al, 2007a; 2007b, 2008).

CONCLUSIONS

A light-weight portable diagnostic sensory based testing device has been successfully designed and fabricated. Protocols designed to enable assessment of systemic alterations to CNS have been implemented and proof of concept has been established by applying the methods to multiple subject populations with a wide spectrum of neurological disorders (neurodevelopmental, neurodegenerative, pharmacological and trauma induced). The potential benefit of such a diagnostic device to the war fighter could be quite significant. Quantitative dosimetry of exposure to blast-related trauma would allow commanders to protect personnel from excessive exposure by reassignment as appropriate in each individual case. Personnel could be assessed for sub-clinical manifestations of TBI as a part of post-blast exposure screening & debriefing. If environmental and medical interventions are set in place, the system would allow quantitative tracking of the efficacy of treatment. Collateral benefits to society could include assessment of cumulative TBI and recovery during medical treatment related to sports injuries, vehicle trauma, developmental and aging disorders, behavioral and nutritional effects on cognitive function. New methods - such as those described in this report – could: enable development of standardized diagnostic criteria of the injury, advance the understanding of threshold of injury and the role of repeated exposure, and identify individual factors that may allow for risk prevention of subjects from over-exposure to such blast injuries. These methods could also provide a means for determination of baseline neuropsychological assessments for the study of the various groups at risk of TBI.

ACKNOWLEDGMENTS

This work was supported, in part, by the Department of Defense CDMRP (W81XWH-07-1-0287) and the Army Research Office (W911NF0810308).

REFERENCES

Bredfeldt, C.E. & Ringach, D.L. (2002). Dynamics of spatial frequency tuning in macaque V1. J Neurosci, 22, 1976-84.

Brumberg, J.C., Pinto, D.J., & Simons, D.J. (1996). Spatial gradients and inhibitory summation in the rat whisker barrel system. J Neurophysiol, 76, 130-40.

Cannestra, A.F., Pouratian, N., Shomer, M.H., & Toga, A.W. (1998). Refractory periods observed by intrinsic signal and fluorescent dye imaging. J Neurophysiol, 80, 1522-32.

Celebrini, S., Thorpe, S., Trotter, Y., & Imbert, M. (1993). Dynamics of orientation coding in area V1 of the awake primate. Vis Neurosci, 10, 811-25.

Chiu, J.S., Tommerdahl, M., Whitsel, B.L., & Favorov, O.V. (2005). Stimulus-dependent spatial patterns of response in SI cortex. BMC Neurosci, 6, 47

Das, A. & Gilbert, C.D. (1995). Receptive field expansion in adult visual cortex is linked to dynamic changes in strength of cortical connections. J Neurophysiol, 74, 779-92.

DeAngelis, G.C., Anzai, A., Ohzawa, I., & Freeman, R.D. (1995). Receptive field structure in the visual cortex: does selective stimulation induce plasticity? Proc Natl Acad Sci U S A, 92, 9682-6.

Derdikman, D., Hildesheim, R., Ahissar, E., Arieli, A., & Grinvald, A. (2003). Imaging spatiotemporal dynamics of surround inhibition in the barrels somatosensory cortex. J Neurosci, 23, 3100-5.

Dinse, H.R. & Kruger, K. (1990). Contribution of area 19 to the foreground-background-interaction of the cat: an analysis based on single cell recordings and behavioural experiments. Exp Brain Res, 82, 107-22.

Folger SE, Tannan V, Zhang Z, Holden JK, and Tommerdahl M. (2008) Effects of the N-methyl-D-Aspartate receptor antagonist dextromethorphan on vibrotactile adaptation. BMC Neuroscience, 2008 Sep 16;9:87.

Foeller, E., Celikel, T., & Feldman, D.E. (2005). Inhibitory sharpening of receptive fields contributes to whisker map plasticity in rat somatosensory cortex. J Neurophysiol, 94, 4387-400.

Francisco EM, Tannan V, Zhang Z, Holden JK, and Tommerdahl M (2008) Vibrotactile amplitude discrimination capacity parallels magnitude changes in somatosensory cortex and follows Weber's Law. Exp Brain Res. 2008 Oct;191(1):49-56. Epub 2008 Jul 24.

Goble, A.K. & Hollins, M. (1993). Vibrotactile adaptation enhances amplitude discrimination. J Acoust Soc Am, 93, 418-24

Grinvald, A. (1985). Real-time optical mapping of neuronal activity: from single growth cones to the intact mammalian brain. Annu Rev Neurosci, 8, 263-305.

Grinvald, A., Frostig, R.D., Siegel, R.M., & Bartfeld, E. (1991). High-resolution optical imaging of functional brain architecture in the awake monkey. Proc Natl Acad Sci U S A, 88, 11559-63.

Kohn, A. (2007). Visual adaptation: physiology, mechanisms, and functional benefits. J Neurophysiol, 97, 3155-64.

Kohn, A., Metz, C., Quibrera, M., Tommerdahl, M.A., & Whitsel, B.L. (2000). Functional neocortical microcircuitry demonstrated with intrinsic signal optical imaging in vitro. Neuroscience, 95, 51-62.

Kohn, A. & Whitsel, B.L. (2002). Sensory cortical dynamics. Behav Brain Res, 135, 119-26.

Moore, C.I., Nelson, S.B., & Sur, M. (1999). Dynamics of neuronal processing in rat somatosensory cortex. Trends Neurosci, 22, 513-20.

Pack, C.C. & Born, R.T. (2001). Temporal dynamics of a neural solution to the aperture problem in visual area MT of macaque brain. Nature, 409, 1040-2.

Pettet, M.W. & Gilbert, C.D. (1992). Dynamic changes in receptive-field size in cat primary visual cortex. Proc Natl Acad Sci U S A, 89, 8366-70.

Ringach, D.L., Hawken, M.J., & Shapley, R. (1997). Dynamics of orientation tuning in macaque primary visual cortex. Nature, 387, 281-4.

Shevelev, I.A., Eysel, U.T., Lazareva, N.A., & Sharaev, G.A. (1998). The contribution of intracortical inhibition to dynamics of orientation tuning in cat striate cortex neurons. Neuroscience, 84, 11-23.

Shevelev, I.A., Volgushev, M.A., & Sharaev, G.A. (1992). Dynamics of responses of V1 neurons Neuroscience, 51, 445-50.

Simons, S.B., Chiu, J., Favorov, O.V., Whitsel, B.L., & Tommerdahl, M. (2007). Duration-dependent response of SI to vibrotactile stimulation in squirrel monkey. J Neurophysiol, 97, 2121-9.

Simons, S.B., Tannan, V., Chiu, J., Favorov, O.V., Whitsel, B.L., & Tommerdahl, M. (2005). Amplitude-dependency of response of SI cortex to flutter stimulation. BMC Neurosci, 6, 43.

Sugase, Y., Yamane, S., Ueno, S., & Kawano, K. (1999). Global and fine information coded by single neurons in the temporal visual cortex. Nature, 400, 869-73.

Tannan, V., Holden, J.K., Zhang, Z., Baranek, G., & Tommerdahl, M.A. (2008). Perceptual metrics of individuals with autism provide evidence for disinhibition., Autism Research. Aug; 1(4): 223-30.

Tannan, V., Simons, S., Dennis, R.G., & Tommerdahl, M. (2007a). Effects of adaptation on the capacity to differentiate simultaneously delivered dual-site vibrotactile stimuli. Brain Res., Jun 18; 1154:116-23.

Tannan V, Dennis RG, Zhang Z, and Tommerdahl M. (2007b) A portable tactile sensory diagnostic device. J Neurosci Methods, Aug 15; 164(1): 131-8.

Tannan, V., Whitsel, B.L., & Tommerdahl, M.A. (2006). Vibrotactile adaptation enhances spatial localization. Brain Res, 1102, 109-16.

Tannan V, Dennis RG, Tommerdahl M (2005a) A novel device for delivering two-site vibrotactile stimuli to the skin. J Neurosci Methods June.

Tannan V, Dennis R, Tommerdahl M (2005b) Stimulus-dependent changes in spatial acuity. Behavioral and Brain Functions Oct 10.

Tommerdahl, M., Favorov, O., & Whitsel, B.L. (2002). Optical imaging of intrinsic signals in somatosensory cortex. Behav Brain Res, 135, 83-91.

Tommerdahl, M., Tannan, V., Cascio, C.J., Baranek, G.T., & Whitsel, B.L. (2007a). Vibrotactile adaptation fails to enhance spatial localization in adults with autism. Brain Res, 1154, 116-23.

Tommerdahl, M., Tannan, V., Zachek, M., Holden, J.K., & Favorov, O.V. (2007b). Effects of stimulus-driven synchronization on sensory perception. Behav Brain Funct, 3, 61.

Tommerdahl M, Tannan V, Holden JK, Baranek GT. (2008) Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? Behavioral and Brain Functions, 4:19.

Tommerdahl, M. & Whitsel, B.L. (1996). Optical imaging of intrinsic signals in somatosensory cortex. In: O. Franzen, R. Johansson, L. Terenius (Eds.). Somesthesis and the Neurobiology of Somatosensory Cortex (pp. 369-384). Basel, Switzerland: Birkhauser Verlag.

Tommerdahl, M., Whitsel, B.L., Favorov, O.V., Metz, C.B., & O'Quinn, B.L. (1999). Responses of contralateral SI and SII in cat to same-site cutaneous flutter versus vibration. J Neurophysiol, 82, 1982-92. PMID: 10515988

Whitsel BL, Favorov OV, Delemos KA, Lee CJ, Tommerdahl M, Essick GK, Nakhle B (1999) SI neuron response variability is stimulus-tuned and NMDA receptor-dependent. Journal of Neurophysiology 81:2988-3006.

Whitsel B, Kelly E, Delemos K, Xu M, Quibrera P (2000) Stability of rapidly adapting afferent entrainment vs responsivity. Somatosensory Motor Research:13-31.

Whitsel BL, Kelly EF, Quibrera M, Tommerdahl M, Li Y, Favorov OV, Xu M, and Metz CB. (2002) Time-dependence of SI RA neuron responses to cutaneous flutter stimulation. Somatosensory & Motor Research, 18: 263-285.

Wirth, C. & Luscher, H.R. (2004). Spatiotemporal evolution of excitation and inhibition in the rat barrel cortex investigated with multielectrode arrays. J Neurophysiol, 91, 1635-47.

Zhang Z, Francisco E, Holden JK, Dennis RG, Tommerdahl M (2009) The impact of non-noxious heat on tactile information processing. Brain Res. 2009 Dec 11;1302:97-105. Epub 2009 Sep 16.

Zhang Z., Zolnoun D., Francisco E., and Tommerdahl M (2010) Differentiating acute and chronic pain in subjects with VVS. *In Submission.*

ARTICLE
An Undergraduate Laboratory Exercise to Study Weber's Law

Jameson K. Holden, Eric M. Francisco, Zheng Zhang, Cristina Baric & Mark Tommerdahl
Biomedical Engineering Department, University of North Carolina, Chapel Hill, NC 27599.

Weber's Law describes the relationship between actual and perceived differences in stimulus intensity. To observe the relationship described in this law, we developed an exercise for undergraduate students, as experiential learning is an integral part of scientific education.

We describe the experimental methods used for determining the subject's discriminative capacity at multiple vibrotactile amplitudes. A novel four-point stimulator (designed and fabricated at the University of North Carolina) was used for the study. Features of the device, such as automated skin detection, make it feasible to perform this laboratory exercise in a reasonable lab period.

At the conclusion of the lab exercise, students will thoroughly understand the principle of Weber's Law as well as fundamental quantitative sensory testing concepts. This introduction to sensory testing will provide a suitable foundation for the undergraduate neuroscience student to investigate other aspects of sensory information processing in subsequent lab exercises.

Keywords: Weber's Law; vibrotactile amplitude discrimination; just noticeable difference

The perceived intensity of a sensory stimulus relative to other stimuli is often difficult to quantify; a subject cannot easily tell whether one stimulus felt twice, half, or three quarters as strong as another. Nevertheless, it is easy to determine which of two stimuli is stronger, provided that the difference between the stimulus intensities is sufficiently large. The minimum physical difference that the subject can perceive -- the just noticeable difference (JND) or difference limen (DL) -- can be measured (Geschieder, 1991).

Ernst Heinrich Weber took advantage of the quantifiable nature of the DL in his 1834 study of perceived intensity. In his experiments he found the DL of blindfolded subjects by giving them two weights of equal magnitudes (standard weight) to hold in each hand. He then proceeded to add slightly heavier weights (test weight) to one hand. The subject was asked to compare the weights in both hands and determine which was larger. Weber found that it was more difficult for the subject to determine that there was a difference in the weights when the standard weight was larger; the size of the DL was proportional to the stimulus strength and increased linearly as the initial weight increased (Goldstein, 2002).

Based on Weber's experiments, physicist Gustav Theodor Fechner developed Weber's law: $\frac{\Delta S}{S} = K$, where ΔS is the difference limen (DL) corresponding to the reference stimulus S, and K is a constant called Weber's Fraction. By publishing this finding in 1860 in the text *Elemente der Psychophysik,* Fechner became the father of the branch of psychology coined 'psychophysics.' Research has shown that Weber's Fraction is constant for a range of stimulus intensities and can be applied to most senses, including touch, sight, and hearing (Formankiewicz and Mollon, 2009; Pienkowski and Hagerman, 2009).

This laboratory exercise was designed for undergraduate students to study Weber's law and its applications to sensory tactile stimulation through the determination of DLs at varying stimulus strengths (Francisco et al., 2008). Each test will consist of delivering a standard and a test sinusoidal vibrotactile stimulus simultaneously to the index and middle fingers of the right hand, after which the subject will choose which stimulus felt more intense. A series of these trials will be carried out, with the objective of determining the subject's difference limen. Students will use the Cortical Metrics Stimulator (CM-4; Cortical Metrics, LLC; Tannan et al., 2007) for these tests. It is an ideal tool for such an experiment as it can deliver up to four vibrotactile stimuli simultaneously, eliminating the need for memory in comparing sequentially delivered stimuli. It also has features such as automatic skin detection that add to the ease and speed with which the lab may be carried out.

LEARNING OBJECTIVES

1. After completion of the experiment, students should have a fundamental understanding of Weber's Law and its applications to tactile stimuli.
2. They should be able to describe the relationship between the physical intensity of a stimulus and perceived intensity.
3. They should understand the advantages of using larger sample sizes as opposed to smaller ones.
4. They should be familiar with the operation procedures of the Cortical Metrics Stimulator as well as fundamentals of sensory data collection and analysis.

MATERIALS AND METHODS

Materials

The Cortical Metrics CM-4 four-point vibrotactile stimulator (Figure 1) was used to conduct the exercise. It interfaced with a personal computer (laptop) through an internal data acquisition box (DAQ) made by National Instruments (NI DAQ USB-6259). The DAQ connects to the computer through a USB cable. The interface software was developed using Microsoft's .NET Framework v3.5. All

computers were networked together to allow centralized data storage in the Cortical Metrics Neurosensory Diagnostics Database. This allowed for instant on-line data analysis at the end of the exercise.

Figure 1. Cortical Metrics (CM-4) Stimulator. INSET: Subject's hand properly positioned on the stimulator.

Procedures

One of the subject's hands was placed on the ergonomic armrest, while the other hand was used to press one of two buttons, located on a response device (mouse) connected directly to the PC.

One trial consisted of the delivery of two simultaneous vibrotactile stimuli, each through independent probe tips, for the duration of one half of a second. These stimuli consisted of 25 Hz sinusoidal vibrations of the probe tips at protocol-specified amplitudes. One of the two stimuli, the standard, was delivered at a constant amplitude throughout a run. The other stimulus, the test, was delivered at amplitudes that were always greater than the amplitude of the standard, but were otherwise varied according to the tracking method used. The digit locations of both the test and standard stimuli were assigned randomly by the computer. The subject responded as to which stimulus felt more intense by clicking the left or right button on the response device, assigned respectively to the left or right digit.

A modified Békésy method, also known as the staircase or up and down method, was used to track subject performance (Cornsweet, 1962). The Békésy method is an adaptive tracking method in which each test stimulus amplitude depends on both the preceding test stimulus amplitude and the subject's response. In this particular experiment, two variations on the Békésy method were used. At the beginning of a run, tracking was conducted with a bias of one: a correct identification of the greater amplitude stimulus lead to a decrease in the test amplitude by a specified step size and an incorrect answer lead to an increase in test amplitude by the same step size. Later in the run (after 10 trials) a bias of two was added to the tracking method; a subject had to deliver two consecutive correct answers for the test amplitude to decrease by one step size, while one incorrect answer lead to an increase in test amplitude by one step size. The bias of one for the first ten trials allowed for the subject to track down quickly, while later increasing the bias to two, increases the accuracy of the results of the run by decreasing the effects of good guessing (Tannan et al, 2006). The method stopped after 20 completed trials. During a run a subject was able to track down to the smallest test amplitude which he/she could consistently differentiate from the standard, that subject's discrimination threshold (JND).

For each run, the test amplitude started at twice the standard amplitude. The step size at which the test amplitude was increased or decreased was 10% of the standard stimulus (e.g., 0.02 mm for a 0.20 mm standard). These settings allowed the test stimulus strength to start out well above the discrimination threshold, but low enough for the subject to track down to his/her JND within the twenty trials that were administered during the run: experience has shown that most subjects can reach their discrimination threshold within ten to fifteen trials.

The standard amplitudes for each run were as follows: 0.2, 0.4, 0.6, 0.8 mm. Maximum and minimum amplitudes were user specified in the protocol so that the stimulator only delivered amplitudes within its proper operating range (0-2mm). The maximum amplitude was set to 3X the standard, while the minimum amplitude was set to the standard amplitude plus 5um. For example, for the first test (0.2 mm standard and 0.40 mm test) the minimum amplitude was 0.205 mm.

After each run was completed, the program generated a graph of the test amplitude versus trial number (Figure 2). It also displayed the discrimination threshold. From previous experience it was determined that most subjects reach their discrimination threshold by the last five trials of a test; therefore the average of the last five test amplitudes is used as the discrimination threshold. The subject's discrimination threshold was determined for each test, and data was subsequently collected from all students in the class (n=11).

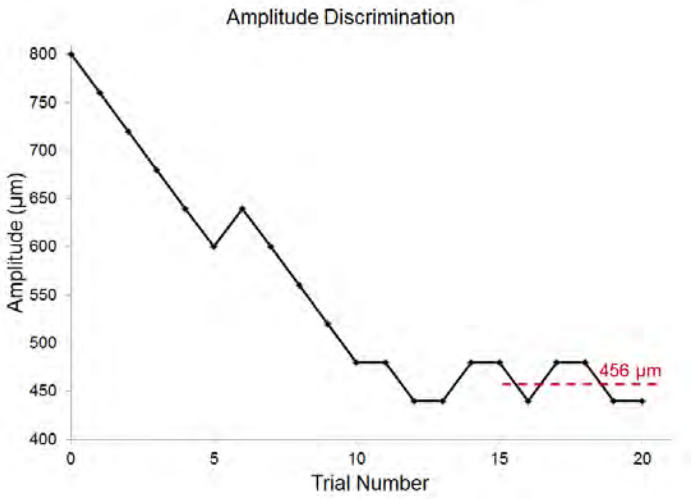


Figure 2. Example program output after completion of a single test run (Standard = 400 µm).

The difference limen (DL) for each subject was determined by subtracting the standard amplitude from its corresponding final test amplitude for each run. The averages of the DL at each standard amplitude were determined for a sub-group of five students as well as for the entire class. These averages were useful in teaching about the effects of sample size. The students then compared their own DL (n=1) with the average DLs for the

group of selected sub-group five people (n=5), as well as the entire class (n=11). Separate plots of DL versus standard amplitude were then constructed for each group. Students compared the three different graphs in order to evaluate the effects of increasing the sample size on experimental results. Students also considered the general form of these graphs and verified that it was in accordance with the linear relationship predicted by Weber's law.

RESULTS AND DISCUSSION

A sample plot for the group of 11 students is shown below (Figure 3). The data we collected from this group of 11 students supported the linear relationship between the difference limen (*ΔS)* and the standard amplitude (*S)* predicted by Weber's Law, and plotting this data resulted in a nearly linear graph, with a linear correlation coefficient of 0.994. The data from the group of five students along with the data from a single individual were still roughly linear, but much less so, with linear correlation coefficients of 0.935 and 0.794, respectively. Clearly, larger sample sizes gave a better approximation of Weber's Law than smaller sample sizes.

Discussion

The exercise was designed to introduce students to Weber's Law using the CM-4 Stimulator as a tool in quantitative testing and measuring of sensory perception. Weber's Law was selected as the subject of this lab because it provides an easily understandable concept as well as a simple protocol for students to test. The CM-4 stimulator was the instrument of choice due to its particular suitability to the task at hand, as well as its versatility, making it a particular valuable research tool to understand. The CM-4 stimulator has a wide range of applications in the area of tactile sensory testing. Simple protocols and portability not only make it ideal for an undergraduate lab setting but for clinical testing and research as well. The integrated software makes it possible to execute protocols that can be adjusted and applied without constant human intervention. The automatic skin detection and programmable test parameters that enable precise control of the amplitude and frequency of stimuli allow for reproducible protocols and reduction of sources of error.

By the end of the exercise paper, students gained a conceptual understanding of Weber's law and its application to sensory testing. Students saw the value of larger experimental sample sizes as well as becoming familiar with the fundamentals of tactile sensory data collection and analysis. The linearity of the collected data is easy to understand, and since linearity in any biological study is rare, we view this result as significant and robust, especially given that it can be collected in a classroom/laboratory setting.

To verify that students accomplished the learning objectives laid out in the introduction of this paper, the instructor may elect to distribute a short quiz. The assessment should be short, and could consist of short-answer questions/problems such as the four below:

1. Define Weber's Law.
2. Provide an example of how Weber's Law relates to tactile perception.
3. Design an experiment to test Weber's Law.
4. When empirically evaluating a scientific hypothesis, is a large or small sample size preferred? Why?

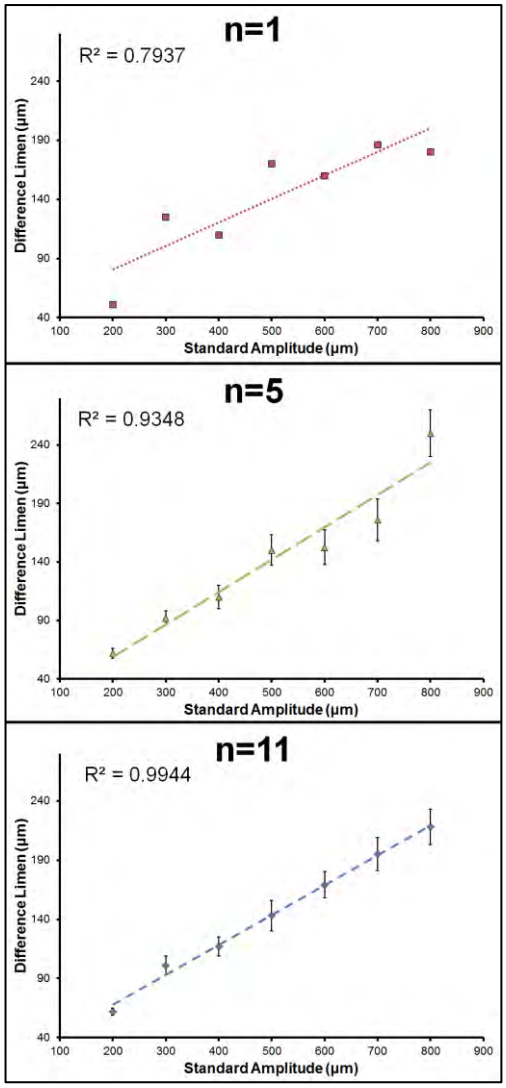


Figure 3. Graph of Difference Limen vs. Standard Amplitude for each sample set (n=1,5, and 11). Error bars at each point represent the standard error.

REFERENCES

Cornsweet TN (1962) The staircase-method in psychophysics. Am J Psychol 75:485-491.

Fechner GT, Elemente der Psychophysik. 1860.

Francisco EM, Tannan V, Zhang Z, Holden JK, Tommerdahl M (2008) Vibrotactile amplitude discrimination capacity parallels magnitude changes in somatosensory cortex and follows Weber's Law. Exp Brain Res:49-56. Epub 2008 Jul 24.

Formankiewicz MA, Mollon JD (2009) The psychophysics of detecting binocular discrepancies of luminance. Vision Res 49:1929-1938. Epub 2009 May 19.

Gescheider GA (1991) Psychophysics: The fundamentals. Mahwah, NJ: Lawrence Erlbaum Associates.

Goldstein, EB (2002) Sensation and Perception. Belmont, CA: Wadsworth Group.

Pienkowski M, Hagerman B (2009) Auditory intensity discrimination as a function of level-rove and tone duration in

normal-hearing and impaired subjects: the "mid-level hump" revisited. Hear Research. 253:107-15. Epub 2009 Apr 2.
Tannan V, Whitsel B, and Tommerdahl M (2006) Vibrotactile adaptation enhances spatial localization. Brain Res 1102:109-116
Tannan V, Dennis RG, Zhang Z, and Tommerdahl M (2007) A portable tactile sensory diagnostic device. J Neurosci Methods 164:131-138.

Received October 06, 2010; revised December 07, 2010; accepted November 23, 2011.

This work was supported, in part by the Department of Defense, Congressionally Directed Medical Research Program (CDMRP W81XWH-07-1-0287) and NIH 5R90DA023417-03.

Address correspondence to: Dr. Mark Tommerdahl, Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599. Email: mark_tommerdahl@med.unc.edu

ORIGINAL ARTICLE

Altered Central Sensitization in Subgroups of Women With Vulvodynia

Zheng Zhang, MS, Denniz A. Zolnoun, MD, MPH,† Eric M. Francisco, BS,* Jameson K. Holden, BS,* Robert G. Dennis, PhD,* and Mark Tommerdahl, PhD**

Objective: To investigate the clinical correlates of central nervous system alterations among women with vulvodynia. Altered central sensitization has been linked to dysfunction in central nervous system-inhibitory pathways (eg, γ-aminobutyric acidergic), and metrics of sensory adaptation, a centrally mediated process that is sensitive to this dysfunction, could potentially be used to identify women at risk of treatment failure using conventional approaches.

Methods: Twelve women with vulvodynia and 20 age-matched controls participated in this study, which was conducted by sensory testing of the right hand's index and middle fingers. The following sensory precepts were assessed: (1) vibrotactile detection threshold; (2) amplitude discrimination capacity (defined as the ability to detect differences in intensity of simultaneously delivered stimuli to 2 fingers); and (3) a metric of adaptation (determined by the impact that applying conditioning stimuli have on amplitude discriminative capacity).

Results: Participants did not differ on key demographic variables, vibrotactile detection threshold, and amplitude discrimination capacity. However, we found significant differences from controls in adaptation metrics in 1 subgroup of vulvodynia patients. Compared with healthy controls and women with a shorter history of pain [n = 5; duration (y) = 3.4 ± 1.3], those with a longer history [n = 7; duration (y) = 9.3 ± 1.4)] were found to be less likely to have adaptation metrics similar to control values.

Discussion: Chronic pain is thought to lead to altered central sensitization, and adaptation is a centrally mediated process that is sensitive to this condition. This report suggests that similar alterations exist in a subgroup of vulvodynia patients.

Key Words: vulvodynia, central sensitization, adaptation

(*Clin J Pain* 2011;00:000–000)

Vulvodynia is a heterogeneous family of idiopathic pain disorders affecting upward of 16% of reproductive age women in the US.[1] It is characterized by both provoked and unprovoked pain in and surrounding vulvar skin, mucosa, and underlying musculature. Clinically, vulvodynia is classified into subgroups based on anatomic location (vulvar mucosa vs. hairy/nonhairy epithelium) and temporal characteristics as provoked versus unprovoked. Although a given patient may experience both provoked and unprovoked pain, the most common complaint is that of provoked pain on contact, precipitated by tampon use or intercourse. Unlike unprovoked pain, where the clinical examination is nonspecific, the majority of women with provoked pain have localized tenderness in vulvar mucosa (ie, vestibule).[2] In addition, women with provoked vulvodynia tend to be younger, and in most instances unaware of their condition until coital debut or the first attempt at using a tampon.

Although both peripheral and central abnormalities have been implicated in vulvodynia, the extent to which peripheral versus central factors contribute to the pain state in an individual patient remains unknown. A substantial portion of women with vulvodynia show hypersensitivity at extragenital sites (eg, arms and feet); this nonspecific hypersensitivity has conventionally been attributed to changes in "central sensitization" caused by the chronic pain state. To date, clinical signs and symptoms associated with central dysregulation in subgroups of women with vulvodynia remains unknown. Thus, understanding of the mechanistic (central vs. peripheral) implication of clinical signs and symptoms in vulvodynia is a necessary first step toward individualized, symptom-based treatment approach.

Current literature[1,3,4] suggests that symptoms of vulvodynia are likely to be triggered by peripheral factors in the skin and underlying musculature. With time (and chronicity), varying degrees of central dysregulation may develop. In this setting, patients may experience superimposed unprovoked (spontaneous) pain in otherwise unaffected tissue. Thus, investigating clinical correlates of central involvement in vulvodynia (eg, how sensory information processing is altered) may provide us with a unique opportunity to investigate the mechanisms of clinically similar disorders (eg, localized pain at the vulvar vestibule vs. generalized vulvar pain). Once the fundamental mechanisms of the centrally versus peripherally mediated vulvar pain is understood, this knowledge will enable the development of robust research and clinical tools that could improve diagnosis and lead to informed therapeutic options.

In this study, we investigated sensory information processing in subgroups of patients with vulvodynia and healthy controls. The quantitative sensory testing methodology used in this study has been shown to be sensitive to systemic cortical alteration,[5–7] and in pilot studies, has been shown to return to normative values with treatment (Tommerdahl; personal communication, 2010). In this study, we hypothesized that women who had experienced a longer time course with pain or had unprovoked symptoms are more likely to have measures consistent with altered central sensitization when compared with healthy controls or those participants who had experienced a shorter duration of provoked pain.

Received for publication December 9, 2010; accepted March 27, 2011.
From the *Department of Biomedical Engineering, University of North Carolina School of Medicine; and †Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC.
This study was supported, in part, by the Department of Defense CDMRP (W81XWH-07-1-0287), NIH/NINDS (PO1 NS045685-061A), and NIH/NICH (K23 HD 053631).
Reprints: Mark Tommerdahl, PhD, 056 MacNider, UNC Chapel Hill, Chapel Hill, NC (e-mail: mark_tommerdahl@med.unc.edu).

MATERIALS AND METHODS

In this study, a convenience sample of 12 women with vulvodynia and 20 healthy controls without gynecologic pain were recruited from the University of North Carolina, Pelvic Pain Clinic and the surrounding community, respectively. The groups did not differ in basic demographic characteristics. All the participants were naive both to the study design and issue under investigation. The study was performed in accordance with the Declaration of Helsinki, all participants gave their written informed consent, and the experimental procedures were reviewed and approved in advance by an institutional review board.

Experimental sessions were conducted with the patients seated comfortably in a chair with the right arm resting on an arm rest attached to the head unit of a portable 4-site vibrotactile stimulator (Fig. 1; CM4; Cortical Metrics, LLC). Vibrotactile stimulation was conducted through 5-mm probes that come in contact with patient's digit 2 (index finger) and digit 3 (middle finger). Glabrous pads of digit 2 (D2) and digit 3 (D3) were chosen as the test sites for 2 reasons: (1) to allow the convenience of access and comfort of the patient, and (2) because of the wealth of neurophysiologic information that exists for the corresponding somatotopic regions of cortex in primates. The independent probe tips are computer controlled and capable of delivery of a wide range of vibrotactile stimulation of varying frequencies (measured in Hertz, Hz) and amplitudes (measured in micrometers, μm). Stimulus parameters are specified by test algorithms that are based on specific protocols and patients' responses during those protocols.

Participants viewed a computer monitor which provided continuous visual cueing during the experimental session. Specifically, an onscreen light panel indicated to the participant when the stimulus was on and when the participant was to respond. Practice trials were performed before each test which allowed the participant to become familiar with the tests, and correct responses on 5 consecutive training trials were required before commencing with each test. The participant was not given performance feedback or knowledge of the results during data acquisition.

FIGURE 1. Images of the multisite vibrotactile stimulator. Stimulators are positioned by rotating each of the 4 independently positioned drums to maximize contact between fingers and the stimulator tips. During an experimental session, the patient was seated comfortably in a chair with the right arm resting on the arm rest attached to the head unit of the stimulator. Index and middle finger were positioned for D2 and D3 stimulation.

The sensory testing session was conducted by application of low frequency (25 Hz) vibration to right hand's index and middle finger(s). The protocols, from start to finish, lasted approximately 30 minutes and consisted of the following 5 modules: (1) static detection threshold; (2) dynamic detection threshold; (3) amplitude discrimination between 2 concurrent and stationary stimuli; (4) the impact of single-site adaptation on amplitude discrimination capacity; and (5) dynamic amplitude discrimination. Exemplary use, technical description, and neurobiologic basis of individual modules have been described in detail earlier.[5–10] An overview of the procedures and the earlier published normative findings is provided below.

Static Detection Threshold

Each participant's vibrotactile detection threshold was measured using a 20-trial 2 Alternative Forced Choice (2AFC) tracking protocol (for recent description with this experiment setup, see earlier studies[9–13]). The left panel of Figure 2A shows the schematic of the protocol. During each trial a 25 Hz vibrotactile test stimulus was delivered to either D2 or D3; the stimulus location was randomly selected on a trial-by-trial basis to minimize participant's inattention and distraction. After each vibrotactile stimulus, the patient was prompted to select the skin site [index (D2) vs. middle (D3) finger] that was perceptually larger. After a 5-second delay, based on patient's response, the stimulation was repeated until the completion of the 20 trials. The stimulus amplitude was started at 15 μm and was modified based on the patient's response in the preceding trial. A 1-up/1-down algorithm was used for the purposes of amplitude modification in the first 10 trials. For example, the stimulus amplitude was decreased by 1 μm if the patient's response in the preceding trial was correct. However, it was increased by the same amount if the response was incorrect. After the initial 10 trials, the amplitude was varied using a 2-up/1-down algorithm (2 correct/1 incorrect patient response(s) resulted in a decrement/increment, respectively, in the amplitude of the stimulus). The rationale for using 1-up/1-down algorithm in the first 10 trials was to expedite determination of patient's vibrotactile discriminative range without affecting the results, and this approach has been reported earlier.[6–10,14,15]

Dynamic Detection Threshold

At the beginning of each trial (as shown in Figure 2A, right panel), a delay period which includes no stimulation was applied. Four conditions of delay ~~(nanoseconds)~~ were used, in separate trials: 0, 1.5, 2, and 3 seconds. After the initial delay, a 25 Hz vibrotactile stimulus was delivered to either D2 or D3 (the stimulus location was randomly selected on a trial-by-trial basis). The amplitude of the stimulus was initiated from zero and increased in steps of 2 μm/s. The patient was instructed to indicate the skin site that received the stimulus as soon as the vibration was detected. The patient's detection threshold was calculated as the average of the stimulus amplitude at the time of patient's response ~~(milliseconds)~~.

Amplitude Discrimination at Baseline

Each patient's amplitude discrimination capacity was assessed using a 2AFC tracking protocol that has been described and implemented in a number of earlier studies.[6–10,14,15] As shown in Figure 2B left panel, during

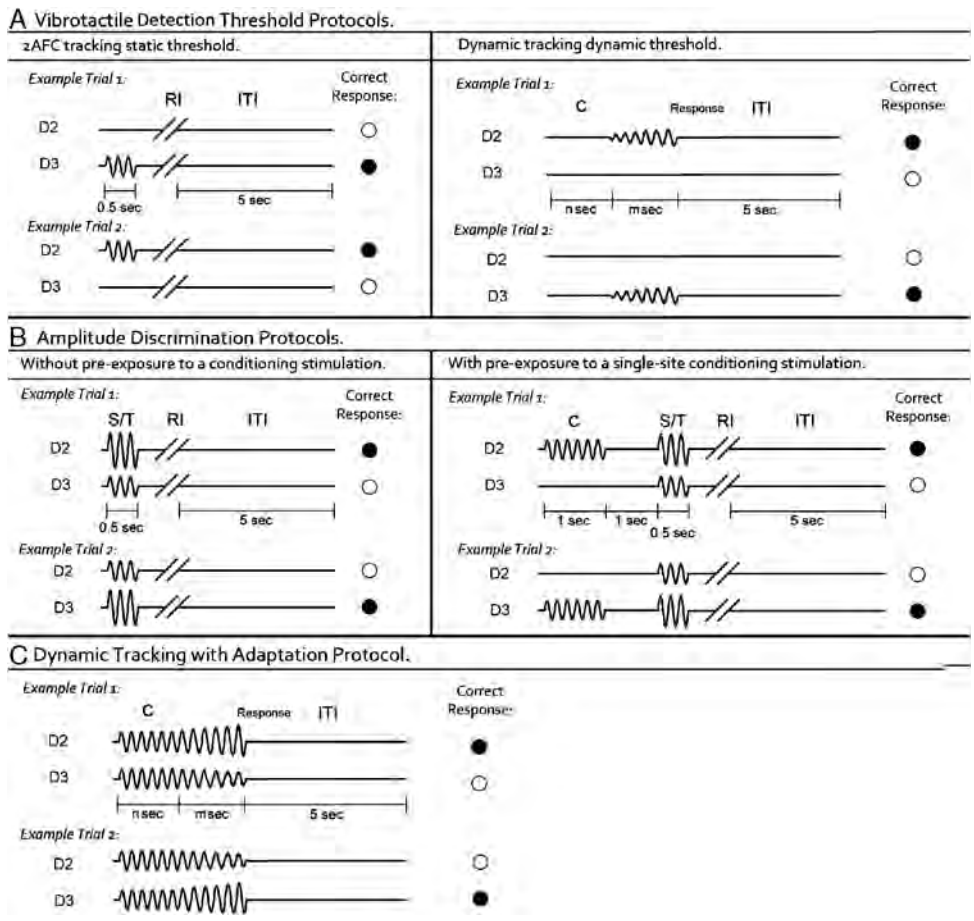


FIGURE 2. Schematics of the protocols used in this study. A, Vibrotactile detection threshold protocols. Left panel: 2 Alternative Forced Choice (2AFC) tracking protocol: In each trial, a 25 Hz vibrotactle test stimulus was delivered to either D2 or D3 for 0.5 second, followed by a patient response interval (RI). Patient was prompted to select the skin site that perceived the stimulus. A 5-second inter-trial interval (ITI) intervened between stimulus response and onset of the next trial. Right panel: Dynamic tracking protocol: A delay period (~~nanosecond~~ = 0, 1.5, 2, or 3 s) without any stimulation was applied. After the initial delay, a 25 Hz vibrotactile stimulus was delivered to either D2 or D3. The amplitude of the stimulus was initiated from zero and increased in steps of 2 μm/s. The stimulation was terminated with patient response to the perceived stimulus. B, Amplitude discrimination protocols. Left panel: Amplitude discrimination at baseline: Two 25 Hz vibrotactile stimuli, the standard (S) and test (T), were delivered simultaneously for 0.5 seconds. Patient was asked to choose the stimulus that was perceptually larger. Right panel: Amplitude discrimination task with preexposure to conditioning stimulation. A 25 Hz conditioning stimulus was delivered 1 second before the presentation of the test and standard stimuli. C, Dynamic tracking with adaptation protocol: Two identical 25 Hz vibrotactile stimuli were delivered simultaneously for a fixed interval (~~nanoseconds~~ = 0, 1.5, 2, or 3 s). After the initial constant stimulus period, the amplitude of the 2 stimuli were dynamically increased/decreased, in steps of 25 μm/s. Stimulation was terminated with patient response to the location at which the most intense stimulus was delivered.

the 20-trial experimental run, a vibrotactile test stimulus (25 Hz, amplitude between 105 and 200 μm) was delivered to ~~1~~ digit pad at the same time that a standard stimulus (25 Hz, amplitude fixed at 100 μm) was applied to the other digit pad. The loci of the test and standard stimuli were randomly selected on a trial-by-trial basis. At the beginning of the experimental run, the test amplitude was 200 μm and the standard amplitude was 100 μm. The difference between the amplitudes of the test and standard stimuli was adjusted on the basis of the patient's response in the preceding trial, such that the difference was decreased/increased after a correct/incorrect response, respectively. The same tracking algorithm as that described for the tactile detection threshold protocol (2AFC tracking protocol) was used to track the participant's ability to determine the most intense stimulus between the test and standard stimuli [ie, the patient's difference limen (DL) was determined]. The step size was held constant at 10 μm throughout the experimental run.

Amplitude Discrimination With Single-site Adaptation

To measure the effects that conditioning stimuli have on subsequent test stimuli, the earlier described amplitude discrimination protocol was modified. As shown in Figure 2B right panel, a 25 Hz 200 μm conditioning stimulus was delivered 1 second before the presentation of the test and standard stimuli. When the conditioning stimulus is delivered to the same site as the test stimulus, the gain effect of adaptation (reducing the perceived intensity) can be quantified by comparison of the amplitude discrimination DL obtained in the adapted versus nonadapted conditions.[6–8,10] The amplitude discrimination tracking algorithm used in the earlier described protocol was used.

Dynamic Amplitude Discrimination

To further characterize the effects of adaptation on amplitude discrimination, a dynamic tracking protocol was implemented (for recent description with this experimental setup, see earlier study[10]). At the start of each run (shown in Figure 2C), 2 vibrotactile stimuli (25 Hz; initially identical in amplitude at 300 μm) were delivered simultaneously to D2 and D3. Four conditions of initial constant stimulus duration (~~nanoseconds~~) were used in separate experimental trials: 0, 1.5, 2, and 3 seconds. After the initial constant or stationary stimulus period, the amplitudes of both stimuli were dynamically altered such that the amplitude of 1 stimulus was increased and the amplitude of the other stimulus was decreased at the rate of 25 μm/s. The participant was instructed to indicate the location at which the most intense stimulus was delivered as soon as the 2 stimuli felt distinctly different in intensity. For each trial, the DL was recorded as the actual difference between the 2 test amplitudes at the time of patient's response (~~milliseconds~~). Averaged DLs were obtained for the 4 different durations of conditioning stimuli that preceded each trial.

Analysis

Repeated-measures analysis of variance was used to evaluate the difference of the patient's performance under different conditions. Data are presented as means and standard errors. A probability of less than 0.05 was considered statistically significant.

RESULTS

This study compared women with vulvodynia and matched healthy controls in a series of sensory perceptual measures that assessed: (1) vibrotactile detection threshold on the fingertip; (2) amplitude discrimination capacity; and (3) the impact of conditioning stimuli on amplitude discrimination capacity. The results show that patients with vulvodynia deviated very little from that of healthy controls in most of the sensory measures obtained in the absence of conditioning stimuli – such as threshold detection and amplitude discriminative capacity, although the patients with vulvodynia demonstrated a tendency to have lower tactile thresholds on the fingertips than controls. Most importantly, the measures of the effects of conditioning stimuli on amplitude discrimination revealed that the patients' data clustered into 2 distinct subgroups (which will be referred to as group A and group B). Group B data was very similar to that obtained from healthy controls, and group A demonstrated a significantly reduced impact of adaptation on the sensory percept. Although the average ages and demographics of the 2 subgroups were not significantly different, there was a significant difference in the duration that the 2 subgroups of patients had pain: group A (n = 7) patients had suffered from vulvodynia for a long duration (average duration: 9.3 ± 1.4 y; average age: 35.7 ± 3.2 y); and group B (n = 5) patients had suffered from vulvodynia for a relatively shorter duration (average duration: 3.4 ± 1.3 y; average age: 34.6 ± 4.3 y).

Patients With Vulvodynia Exhibit Slightly Lower Tactile Detection Thresholds

Figure 3 summarizes the group-averaged detection thresholds. As shown in the left panel of Figure 3, the group-averaged static thresholds observed were 12.37 ± 1.34 μm for controls, 9.77 ± 2.23 μm for patients in group A, and 10.32 ± 1.85 μm for patients in group B. The data suggest an elevated sensitivity for patients with vulvodynia compared with controls, although this difference was not statistically significant (group A vs. controls: $P = 0.35$; group B vs. controls: $P = 0.51$). This finding is consistent with data reported by Pukall et al[16] which showed that women suffering from vulvodynia had a lower tactile threshold than controls at sites distant to the genitalia area.

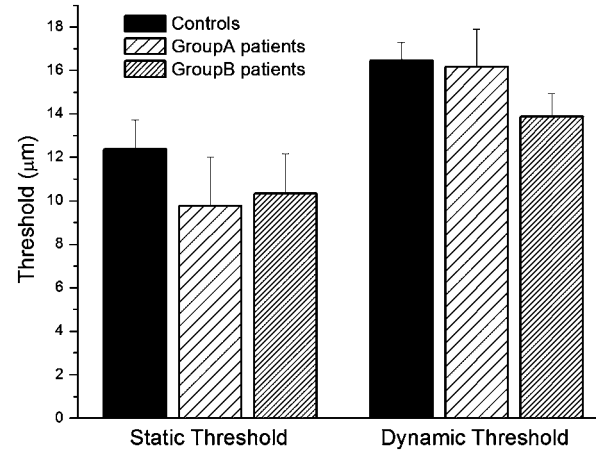


FIGURE 3. Summary of group-averaged vibrotactile detection thresholds obtained with 2 different methods on 2 subgroups of patients with vulvodynia and controls. Static threshold: No significant differences were observed on the static thresholds between any patients group and controls (group A vs. controls: P=0.35; and group B vs. controls: P=0.51). Dynamic threshold: The group-averaged dynamic thresholds of patients with vulvodynia did not significantly differ from that of controls, whereas data from patients in group B show a trend for lower dynamic threshold than controls.

As several studies have reported that psychophysical measurement methods had a significant influence on vibrotactile thresholds,[17,18] in this study, the patient's vibrotactile threshold was also measured by a dynamic tracking protocol. The group-averaged dynamic thresholds are shown in the right panel of Figure 3. There was no significant difference between the controls and 2 vulvodynia patients groups, although data from patients in group B showed a lower (although not statistically significant) dynamic threshold than controls.

Although amplitude discrimination capacity was not significantly different between the controls and patients with vulvodynia, the impact of conditioning stimuli on performance during this task revealed that the vulvodynia patients were clustered into 2 distinct subgroups.

Figure 4 summarizes the group-averaged performance during amplitude discrimination tests for the controls and 2 subgroups of patients with vulvodynia. Weber's fractions (WF) were determined by normalizing each patient's DL to the amplitude of standard stimulus (100 μm). As shown in the left panel of Figure 4, during which amplitude discrimination was measured in the absence of conditioning stimulus, there was no significant difference in performance between the controls and groups of vulvodynia patients. Specifically, control participants were able to discriminate the difference between the test and standard stimuli that is 24.4% of the standard amplitude (WF = 0.244), and the patients in groups A and B were able to discriminate, respectively, 33.5% (WF = 0.335) and 31.6% (WF = 0.316)

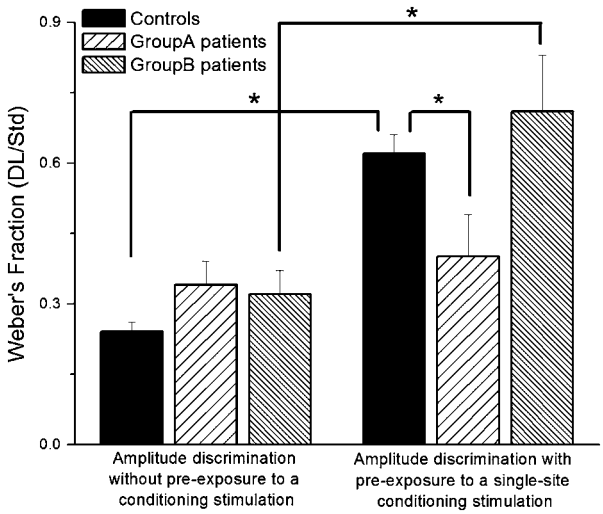


FIGURE 4. Comparison of Weber's fraction obtained with amplitude discrimination protocols (without/with preexposure to a single-site conditioning stimulus). In the absence of conditioning stimulus, no significant difference was observed between the performance of controls and subgroups of vulvodynia patients. Preexposure to a single-site conditioning stimulation (1 s in duration) caused a significant degradation of performance in the controls ($P<0.01$) and the patients in group B ($P=0.017$). In contrast to controls and group B, patients in group A performed equally well under both adapted and unadapted conditions. Under the condition with adaptation, the group-averaged performance is significantly different between controls and group A ($P=0.036$). DL indicates difference limen; Std, standard.

of the standard amplitude. However, preexposure to a single-site conditioning stimulus dramatically changed the patients' performance (shown in Figure 4, right panel). Although the WF of controls and patients in group B is significantly elevated in the adapted condition compared with the unadapted condition, patients in group A performed equally well under both adapted and unadapted conditions. Earlier reports have shown that single-site adaptation impairs control participant's amplitude discrimination capacity.[6–8,10] One interpretation of the impairment observed in this study is that a 1-second conditioning stimulus reduces the perceived intensity of the subsequent test stimulus to the extent that a test stimulus with amplitude of approximately 162% (controls)/171% (group B) of the standard amplitude was perceived nearly the same in intensity as the standard stimulus. Comparing with the significant degradation of performance of the controls ($P<0.01$) and the patients in group B ($P=0.017$) because of adaptation, no change was observed in the patients in group A ($P=0.52$). Moreover, under the adapted condition the group-averaged performance is significantly different between controls and patients in group A ($P=0.036$). Therefore, conditioning stimulation significantly impaired the performance of the controls and the patients in group B, but has no effects on the patients in group A.

To determine whether the differential effects of adaptation observed between groups were consistent within patients, each patient's WF obtained under the adapted condition was normalized to the unadapted condition. As shown in Figure 5, The 1-second conditioning stimulus significantly impaired amplitude discrimination capacity by

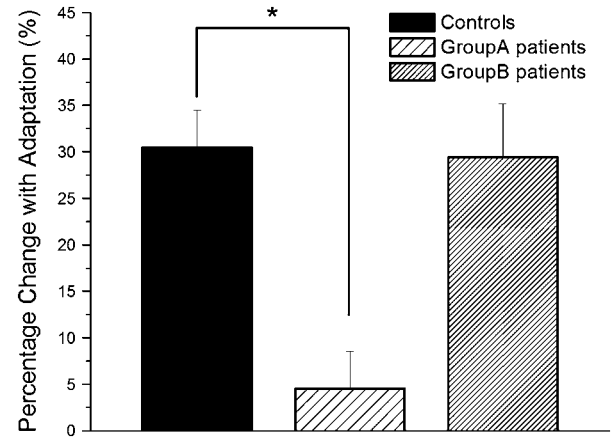


FIGURE 5. Weber's fraction obtained under the condition with adaptation was normalized on a patient-by-patient basis to the unadapted condition. The 1-second conditioning stimulus significantly impaired the patients' amplitude discrimination capacity by nearly 30% for both the controls and the patients in group B, whereas there were much lesser effects (3%) of adaptation observed in the patients in group A.

nearly 30% for both the controls and the patients in group B, whereas there was much less of an effect (3%) of adaptation observed in the patients in group A ($P<0.01$).

Dynamic Amplitude Discrimination

A dynamic amplitude discrimination protocol was used which is able to effectively compare the degree to which a patient adapts to simultaneously delivered dual-site vibrotactile stimuli at different durations of conditioning stimulation. Figure 6 summarizes the group-averaged performance with dual-site adaptation at the 4 different durations of conditioning stimulation (0, 1.5, 2, and 3 s) for

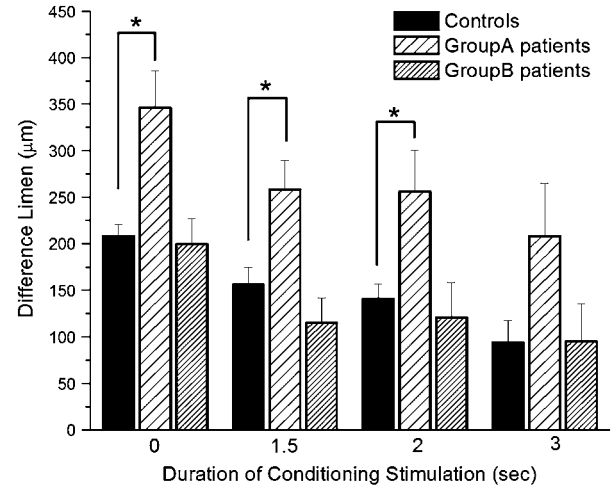


FIGURE 6. Comparison of the group-averaged performance with dual-site adaptation at the 4 different durations of dual-site conditioning stimulation (0, 1.5, 2, and 3 s) for the controls and 2 subgroups of patients with vulvodynia. Increasing the duration of the conditioning stimuli led to an improvement of performance (ie, reduced DL). As the data obtained from patients in group B deviated very little from that of controls, DLs obtained from patients in group A were significantly higher compared with controls and showed only little effect with adaptation. DL indicates difference limen.

the controls and 2 subgroups of patients with vulvodynia. The results show that increasing the duration of the conditioning stimuli delivered to both sites of skin led to an improvement of a patient's capacity to detect the difference in amplitude between the 2 stimuli. For example, after preexposure to 1.5, 2, or 3-second conditioning stimulus, control participants were, on average, able to attain a DL (156, 141, and 94 μm) that was approximately 73%, 66%, or 42% of the DL (208 μm) obtained without adaptation, respectively. Compared with controls, 2 subgroups of patients with vulvodynia have distinct performance differences. Specifically, the DLs were significantly higher in patients of group A compared with controls (0 s adaptation: $P < 0.01$; 1.5 s adaptation: $P = 0.01$; 2 s adaptation: $P < 0.01$; and 3 s adaptation: $P = 0.06$), but there was no significant difference between patients of group B and controls in the DLs obtained under all the conditions. In summary, data obtained from patients in group A showed little effect with conditioning stimulation whereas the data obtained from patients in group B deviated very little from that of controls.

DISCUSSION

In this study, sensory perceptual measures were obtained in 12 patients diagnosed with vulvodynia and 20 healthy controls. Five tests were performed to assess: (1) detection threshold on the fingertips; (2) amplitude discrimination capacity; and (3) the effects of adaptation on tactile discrimination capacity. The results suggest that women with vulvodynia have—although not statistically significantly—lower tactile thresholds on the fingertips than do control participants. Furthermore, as amplitude discrimination capacity was not significantly different between the controls and patients with vulvodynia, the impact of single-site conditioning (or adaptation) on performance of the dual-site task showed a remarkable difference. Specifically, the observations of the conditioned sensory measures revealed that the patients with vulvodynia were clustered into 2 distinct subgroups. Group B had data that was very similar to that obtained from healthy controls, whereas group A demonstrated a significantly reduced impact of adaptation on the sensory percept. The primary difference between the compositions of the 2 subgroups is the duration or longevity of pain of the patients in each subgroup. Group B was composed of patients who reported pain for an average of 3.4 ± 1.3 years, whereas group A was composed of patients who reported pain for an average duration of 9.3 ± 1.4 years.

The reduction of the adaptation metric in patients with vulvodynia studied in this study has not been reported earlier. There have been few studies to date that have assessed the changes in perception that normally result from repetitive vibrotactile stimulation on the population of chronic pain patients, although Hollins and colleagues[19] did report decreased effects of adaptation in patients with temporomandibular disorders. Neurophysiologic studies have shown that repetitive stimulation results in temporal changes of cortical activity, the most prominent of which is a reduction in cortical response with extended stimulus duration. At the single-cell level, both visual and somatosensory cortical pyramidal neurons undergo prominent use-dependent modifications of their receptive fields and response properties with repetitive stimulation. These modifications can attain full development within a few tens of milliseconds of stimulus onset, and can disappear within seconds after the stimulus ends (visual cortical neurons[20–30]; alternatively, for review of short-term cortical neuron dynamics in visual cortex[31]; and for review of short-term primary somatosensory cortical neuron dynamics[32–36]). Optical imaging studies have also characterized the short-term dynamics of the population-level response of squirrel monkey contralateral primary somatosensory cortex using different amplitudes and durations of vibrotactile stimulation.[37–39] Guided by the scientific work mentioned above, our research group has designed a series of tactile sensory diagnostics which effectively assess the impact that adaptation has on perception.[5,7–10,15] For example, the protocols used in this study directly measure the change in amplitude discrimination capacity that occurs with prior conditioning stimuli. Earlier studies using this measure showed that a patient's ability to discriminate between 2 simultaneously delivered vibrotactile stimuli—differing only in amplitude and location—was very robust and repeatable across a large number of healthy controls, but it was also very sensitive to varying conditions of conditioning stimuli. For instance, changing the duration of the conditioning stimulus delivered to 1 of the 2 sites before the amplitude discrimination task significantly altered a patient's ability to determine the actual difference between the 2 stimuli in a predictive and quantifiable manner. As a result, these methods could be viewed as a reliable indicator of the influence of adapting stimuli on central nervous system (CNS) response, as changes in the peripheral response are not significantly changed at these short stimulus durations (for discussion, see Refs.5,8,9,40,41). Centrally mediated adaptation is dependent on several factors [eg, γ-aminobutyric acid (GABA)ergic and *N*-methyl-D-aspartate receptor-mediated neurotransmission, neuron-glial interactions) which play significant roles in the way in which cortical information processing capacities of a number of clinically identified patient populations are impacted by their respective disorder. For example, conditioning stimuli do not have as pronounced impact on the amplitude discriminative capacity of patients with autism as it does with typically developing patients (for discussion of GABA-deficiencies in autism, see Refs.5,6,41). In addition, patients administered with a relatively small dose of an *N*-methyl-D-aspartate receptor antagonist (60 mg of dextromethorphan) also demonstrated a degraded adaptation metric.[7]

Two aspects of the adaptation process were measured in this study. The first, the gain effects of adaptation, was derived from the amplitude discrimination task in which a conditioning stimulus was delivered on 1 of the 2 test sites. The effect of that conditioning stimulus was on the gain of the conditioned site—that site was now perceived to be much smaller and thus, a reduction in gain was manifested, and subsequently, patients (normally) become worse at the task. The second facet of adaptation that was measured was a contrast effect, in which contrast between 2 stimuli improve after conditioning stimuli have been delivered to both of the test sites, and the patients (normally) perform better after conditioning than they do without. In this study, the data obtained from the patients with vulvodynia are clustered into 2 distinct subgroups consistently with both of these aspects of adaptation. The patients in group B performed very similar as healthy controls did, and the performance of the patients in group A showed a significantly reduced impact of conditioning stimulation on the sensory percept. However, other sensory measures

obtained in the absence of conditioning stimuli—such as threshold detection and amplitude discriminative capacity—showed no statistically significant difference between the 2 subgroups. The primary difference between the compositions of the 2 subgroups of note is the duration that patients of the subgroups have had pain, whereas average age of the 2 subgroups was not significantly different. Considering the metrics of adaptation (measuring the effects of conditioning stimulation on sensory perception) could be a reliable indicator of systemic alterations on central nervous function, it is speculated that the performance difference between the 2 subgroups of patients with vulvodynia observed in this study might reflect the level of dysregulation of their CNS due to chronic vulvar pain.

The involvement of both peripheral and central mechanisms in the development and maintenance of vulvodynia has been supported by a series of studies.[3,16,42–47] For example, it has been found that patients with vulvodynia have increased sensitivity to sensory stimulation at both genital regions and sites distant to it.[3,16,44] This suggests that not only peripheral sensitization but also a generalized central abnormality is involved in vulvodynia and could be similar to that observed in patients with other pain syndromes, implying a widespread disturbance in the CNS.[45] The observation of increased tactile sensitivity of the skin area distant to the vulvar region—including the static thresholds of all patients with vulvodynia in this report—is consistent with altered central sensitization that develops with chronic pain.

All participants, including controls, demonstrated a dynamic threshold that was higher than their static threshold. This noticeable difference in the threshold between the 2 tasks is consistent with earlier reports.[10,18] Although this could possibly be explained by the influence that psychophysical measurement methods have on tactile detection,[17,18] we believe an alternate explanation is much more plausible. Mechanistically, this phenomenon could be the result of feed-forward inhibition that is generated by the initial subthreshold stimulus that occurs when the threshold test is ramped from zero to the detectable level.[48] The significance of this is that this type of feed-forward inhibition takes place in somatosensory cortical input layer 4,[49] in which local layer 4 inhibitory cells receive direct thalamocortical input and in turn suppress responses of neighboring layer 4 excitatory cells to their thalamocortical drive, thereby sharpening their ~~RF~~ properties.[50–55] These inhibitory cells are more responsive to weak (near-threshold) afferent drive than are the excitatory layer 4 cells, and thus, subthreshold or weak stimulus inputs will have the effect of raising the threshold at which excitatory layer 4 cells begin to respond to peripheral stimuli. Thus, although not statistically significant, the observation of the difference between the groups A and B patients in their dynamic thresholds is that the difference between the ratio of the respective dynamic and static thresholds are clearly evident, and suggestive of below normal feed-forward inhibition. If this alteration is, as we believe, sensitive to the time dependency of the GABAb receptor, then the measure itself might be an indicator that GABAb efficiency has been compromised in some individuals.

Our data on patients with vulvodynia is consistent with existing constructs in the pain literature and supports the notion that the relative contribution of peripheral and central factors differ in subgroups of women with vulvodynia, and that clinical signs and symptoms alone are insufficient in identifying the underlying mechanism of pain as peripheral, central, or a combination of both. A wide range of therapies for vulvodynia have been proposed that include topical therapies, pharmacologic regimens, physical therapy, surgery, and cognitive behavioral therapy.[56] However, outcomes with these therapies vary widely. For example, as a commonly reported therapy for localized vestibular dysesthesia, vestibulectomy is most effective for a specific subset of patients, specifically for women below 30 years of age who have localized vulvar pain and provoked pain. [57,58] These findings suggest that it is possible that this type of pain represents a localized nociceptor mechanism, whereas unprovoked and generalized pain could have a different mechanism. Our data suggest that women suffering vulvar pain for long duration or with unprovoked pain have more CNS involvement or dysregulation. The CNS involvement occur de novo (eg, genetic polymorphism) or secondary to an intractable pain state; the latter is the likely mechanism by which women with provoked vulvodynia transition into unprovoked or chronic pain state. It is well documented that an intractable peripheral process can lead to neuroplastic changes (through central sensitization) at all levels of the CNS and "generalization of pain."[59]

The findings in this study are consistent with the idea that chronic pain, caused by vulvodynia, alters central sensitization that leads to changes in sensory information processing. These changes are manifested in lower sensory thresholds (or higher sensitivity) in sites without provoked pain—because of a change in the balance between excitation and inhibition (or glutamatergic and gabaergic neurotransmission). Lower thresholds are consistent with this imbalance; decreasing inhibition will result in less suppression of cortical activity. In other words, a simple stimulus on the skin will generate more cortical activity if altered central sensitization has resulted in decreased inhibition or increased excitation. However, threshold testing has not been considered as an efficient method in measuring altered central sensitization because of large inter-individual variability. And to show these small differences, group differences of repeated measurements are normally necessary. Alternatively, using a measure—such as an adaptation metric—in which the patient provides their own individual baseline (ie, the adaptation metric is derived on how amplitude discriminative capacity is impacted by conditioning)—could prove to be a more effective indicator of altered central sensitization that can be obtained reliably and efficiently (protocols used in this study can be obtained within 2 to 3 min). Sensory-based measures of altered central sensitization seem to differentiate chronicity within subgroups of vulvodynia, and future studies will continue to investigate the changes in sensitization that seem to occur with the time course of the history of vulvodynia.

REFERENCES

1. Danby CS, Margesson LJ. Approach to the diagnosis and treatment of vulvar pain. *Dermatol Ther*. 2010;23:485–504.
2. Harlow BL, Wise LA, Stewart EG. Prevalence and predictors of chronic lower genital tract discomfort. *Am J Obstet Gynecol*. 2001;185:545–550.
3. Giesecke J, Reed BD, Haefner HK, et al. Quantitative sensory testing in vulvodyniapatients and increased peripheral pressure pain sensitivity. *Obstet Gynecol*. 2004;104:126–133.
4. Gunter J. Vulvodynia: new thoughts on a devastating condition. *Obstet Gynecol Surv*. 2007;62:812–819.

5. Tommerdahl M, Tannan V, Cascio CJ, et al. Vibrotactile adaptation fails to enhance spatial localization in adults with autism. *Brain Res*. 2007;1154:116–123.
6. Tannan V, Holden J, Zhang Z, et al. Perceptual metrics of individuals with autism provide evidence for disinhibition. *Autism Res*. 2008;1:223–230.
7. Folger SE, Tannan V, Zhang Z, et al. Effects of the *N*-methyl-D-Aspartate receptor antagonist dextromethorphan on vibrotactile adaptation. *BMC Neurosci*. 2008;9:87.
8. Tannan V, Simons S, Dennis RG, et al. Effects of adaptation on the capacity to differentiate simultaneously delivered dual-site vibrotactile stimuli. *Brain Res*. 2007;1186:164–170.
9. Francisco E, Tannan V, Zhang Z, et al. Vibrotactile amplitude discrimination capacity parallels magnitude changes in somatosensory cortex and follows Weber's Law. *Exp Brain Res*. 2008;191:49–56.
10. Zhang Z, Francisco E, Holden JK, et al. The impact of non-noxious heat on tactile information processing. *Brain Res*. 2009;1302:97–105.
11. Tannan V, Dennis R, Tommerdahl M. A novel device for delivering two-site vibrotactile stimuli to the skin. *J Neurosci Methods*. 2005;147:75–81.
12. Tannan V, Dennis GR, Tommerdahl M. Stimulus-dependent effects on tactile spatial acuity. *Behav Brain Funct*. 2005; 1:18.
13. Tannan V, Whitsel BL, Tommerdahl MA. Vibrotactile adaptation enhances spatial localization. *Brain Res*. 2006;1102: 109–116.
14. Tannan V, Dennis RG, Zhang Z. A portable tactile sensory diagnostic device. *J Neurosci Methods*. 2007;164:131–138.
15. Zhang Z, Tannan V, Holden JK. A quantitative method for determining spatial discriminative capacity. *BioMed Eng Online*. 2008;7:12.
16. Pukall CF, Binik YM, Khalife S. Vestibular tactile and pain thresholds in women with vulvar vestibulitis syndrome. *Pain*. 2002;96:163–175.
17. Maeda S, Grinffin MJ. A comparison of vibrotactile thresholds on the finger obtained with different measuring algorithms. ~~Stockholm workshop proceedings of hand-arm vibration syndrome: diagnostics and quantitative relationships to exposure. ■, 1995;94:85–95.~~
18. Morioka M, Griffin MJ. Dependence of vibrotactile thresholds on the psychophysical measurement method. *Int Arch Occup Environ Health*. 2002;75:78–84.
19. Hollins M, Sigurdsson A, Fillingim L, et al. Vibrotactile threshold is elevated in temporomandibular disorders. *Pain*. 1996;67:89–96.
20. Bredfeldt CE, Ringach DL. Dynamics of spatial frequency tuning in macaque V1. *J Neurosci*. 2002;22:1976–1984.
21. Celebrini S, Thorpe S, Trotter Y, et al. Dynamics of orientation coding in area V1 of the awake primate. *Vis Neurosci*. 1993;10:811–825.
22. Das A, Gilbert CD. Receptive field expansion in adult visual cortex is linked to dynamic changes in strength of cortical connections. *J Neurophysiol*. 1995;74:779–792.
23. DeAngelis GC, Anzai A, Ohzawa I, et al. Receptive field structure in the visual cortex: does selective stimulation induce plasticity? *Proc Natl Acad Sci U S A*. 1995;92: 9682–9686.
24. Dinse HR, Kruger K. Contribution of area 19 to the foreground-background-interaction of the cat: an analysis based on single cell recordings and behavioural experiments. *Exp Brain Res*. 1990;82:107–122.
25. Pack CC, Born RT. Temporal dynamics of a neural solution to the aperture problem in visual area MT of macaque brain. *Nature*. 2001;409:1040–1042.
26. Pettet MW, Gilbert CD. Dynamic changes in receptive-field size in cat primary visual cortex. *Proc Natl Acad Sci U S A*. 1992;89:8366–8370.
27. Ringach DL, Hawken MJ, Shapley R. Dynamics of orientation tuning in macaque primary visual cortex. *Nature*. 1997;387:281–284.
28. Shevelev IA, Eysel UT, Lazareva NA, et al. The contribution of intracortical inhibition to dynamics of orientation tuning in cat striate cortex neurons. *Neuroscience*. 1998;84: 11–23.
29. Shevelev IA, Volgushev MA, Sharaev GA. Dynamics of responses of V1 neurons evoked by stimulation of different zones of receptive field. *Neuroscience*. 1992;51: 445–450.
30. Sugase Y, Yamane S, Ueno S, et al. Global and fine information coded by single neurons in the temporal visual cortex. *Nature*. 1999;400:869–873.
31. Kohn A. Visual adaptation: physiology, mechanisms, and functional benefits. *J Neurophysiol*. 2007;97:3155–3164.
32. Kohn A, Whitsel B. Sensory cortical dynamics. *Behav Brain Res*. 2002;135:119–126.
33. Tommerdahl M, Delemos KA, Favorov OV, et al. Response of anterior parietal cortex to different modes of same-site skin stimulation. *J Neurophysiol*. 1998;80:3272–3283.
34. Tommerdahl M, Favorov OV, Whitsel BL. Effects of high-frequency skin stimulation on SI cortex: mechanisms and functional implications. *Somatosens Mot Res*. 2005;22: 151–169.
35. Tommerdahl M, Simons SB, Chiu JS, et al. Response of SII cortex to ipsilateral, contralateral and bilateral flutter stimulation in the cat. *BMC neurosci*. 2005;6:11.
36. Tommerdahl M, Whitsel BL, Vierck CJ Jr, et al. Effects of spinal dorsal column transection on the response of monkey anterior parietal cortex to repetitive skin stimulation. *Cereb Cortex*. 1996;6:131–155.
37. Chiu JS, Tommerdahl M, Whitsel BL, et al. Stimulus-dependent spatial patterns of response in SI cortex. *BMC Neurosci*. 2005;6:47.
38. Simons SB, Chiu J, Favorov OV, et al. Duration-dependent response of SI to vibrotactile stimulation in squirrel monkey. *J Neurophysiol*. 2007;97:2121–2129.
39. Simons SB, Tannan V, Chiu J, et al. Amplitude-dependency of response of SI cortex to flutter stimulation. *BMC Neurosci*. 2005;6:43.
40. Tommerdahl M, Tannan V, Zachek M, et al. Effects of stimulus-driven synchronization on sensory perception. *Behav Brain Funct*. 2007;3:61.
41. Tommerdahl M, Tannan V, Holden JK, et al. Absence of stimulus-driven synchronization effects on sensory perception in autism: evidence for local underconnectivity? *Behav Brain funct*. 2008;4:19.
42. Bergeron S, Binik YM, Khalife S, et al. A randomized comparison of group cognitive–behavioral therapy, surface electromyographic biofeedback, and vestibulectomy in the treatment of dyspareunia resulting from vulvar vestibulitis. *Pain*. 2001;91:297–306.
43. Marinoff SC, Turner ML. Vulvar vestibulitis syndrome: an overview. *Am J Obstet Gynecol*. 1991;165:1228–1233.
44. Bohm-Starke N, Hilliges M, Brodda-Jansen G, et al. Psychophysical evidence of nociceptor sensitization in vulvar vestibulitis syndrome. *Pain*. 2001;94:177–183.
45. Pukall CF, Strigo IA, Binik YM, et al. Neural correlates of painful genital touch in women with vulvar vestibulitis syndrome. *Pain*. 2005;115:118–127.
46. Gordon AS, Panahian-Jand M, Mccomb F, et al. Characteristics of women with vulvar pain disorders: responses to a Web-based survey. *J Sex Marital Ther*. 2003;29:45–58.
47. Zolnoun D, Hartmann K, Lamvu G, et al. A conceptual model for the pathophysiology of vulvar vestibulitis syndrome. *Obstet Gynecol Surv*. 2006;61:395–401.
48. Tommerdahl M, Favorov OV, Whitsel BL. Dynamic representations of the somatosensory cortex. *Neurosci Biobehav Rev*. 2010;34:160–170.
49. Favorov OV, Kursun O. Neocortical layer 4 as a pluripotent function linearizer. *J Neurophysiol*. 2011;105:1342–1360. [Epub ahead of print].
50. Douglas RJ, Koch C, Mahowald M, et al. Recurrent excitation in neocortical circuits. *Science*. 1995;269:981–985.

51. Miller KD, Pinto DJ, Simons DJ. Processing in layer 4 of the neocortical circuit: new insights from visual and somatosensory cortex. *Curr Opin Neurobiol*. 2001;11:488–497.
52. Bruno RM, Simons DJ. Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. *J Neurosci*. 2002;22:10966–10975.
53. Alonso JM, Swadlow H. Thalamocortical specificity and the synthesis of sensory cortical receptive fields. *J Neurophysiol*. 2005;94:26–32.
54. Sun QQ, Huguenard JR, Prince DA. Barrel cortex microcircuits: thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of fast-spiking interneurons. *J Neurosci*. 2006;26:1219–1230.
55. Cruikshank SJ, Lewis TJ, Connors BW. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. *Nat Neurosci*. 2007;10:462–468.
56. Goldstein AT, Marinoff SC, Haefner HK. Vulvodynia: strategies for treatment. *Clin Obstet Gynecol*. 2005;48:769–785.
57. Traas MA, Bekkers RL, Dony JM, et al. Surgical treatment for the vulvar vestibulitis syndrome. *Obstet Gynecol*. 2006;107:256–262.
58. Bornstein J, Goldik Z, Stolar Z, et al. Predicting the outcome of surgical treatment of vulvar vestibulitis. *Obstet Gynecol*. 1997;89:695–698.
59. Woolf CJ, Doubell TP. The pathophysiology of chronic pain-increased sensitivity to low threshold Ab-fibre inputs. *Curr Opin Neurobiol*. 1994;4:525–534.

Author Reprints

For *Rapid Ordering* go to: www.lww.com/periodicals/author-reprints

The Clinical Journal of Pain

Lippincott Williams & Wilkins
a Wolters Kluwer business

Order

Author(s) Name

Title of Article

*Article # *Publication Mo/Yr

**Fields may be left blank if order is placed before article number and publication month are assigned.*

Quantity of Reprints ____ $_____

Covers (Optional) _____ $_____

Shipping Cost $_____

Reprint Color Cost $_____

Tax $_____

Total $_____

REPRINTS ORDERED & PURCHASED UNDER THE AUTHOR REPRINTS PROGRAM MAY NOT BE USED FOR COMMERCIAL PURPOSES

Reprint Pricing

50 copies = $336.00
100 copies = $420.00
200 copies = $494.00
300 copies = $571.00
400 copies = $655.00
500 copies = $732.00

Plain Covers

$108.00 for first 100 copies
$18.00 each add'l 100 copies

Reprint Color

($70.00/100 reprints)

Shipping

Within the U.S. - $15.00 up to the first 100 copies and $15.00 for each additional 100 copies

Outside the U.S. - $30.00 up to the first 100 copies and $30.00 for each additional 100 copies

Tax

U.S. and Canadian residents add the appropriate tax or submit a tax exempt form.

Payment

- MC
- VISA
- Discover
- American Express

Account # / / Exp. Date

Name

Address Dept/Rm

City State Zip Country

Telephone

Signature

Ship to

Name

Address Dept/Rm

City State Zip Country

Telephone

Use this form to order reprints. Publication fees, including color separation charges and page charges will be billed separately, if applicable.

Payment must be received before reprints can be shipped. Payment is accepted in the form of a check or credit card; purchase orders are accepted for orders billed to a U.S. address.

Prices are subject to change without notice.

For quantities over 500 copies contact our Healthcare Dept. For orders shipping in the US and Canada: call 410-528-4396, fax your order to 410-528-4264 or email it to Meredith.Doviak@wolterskluwer.com. Outside the US: dial 44 1829 772756, fax your order to 44 1829 770330 or email it to Christopher.Bassett@wolterskluwer.com.

MAIL your order to:
Lippincott Williams & Wilkins
Author Reprints Dept.
351 W. Camden St.
Baltimore, MD 21201

FAX:
410.528.4434

For questions regarding reprints or publication fees,
E-MAIL:
reprints@lww.com

OR **PHONE:**
1.866.903.6951

For *Rapid Ordering* go to: www.lww.com/periodicals/author-reprints